UNIVERSIDAD AUTÓNOMA DE MADRID
Facultad de Ciencias
Departamento de Biología

Eco-etología y respuestas de estrés fisiológico en el gato montés (*Felis silvestris*): implicaciones para su conservación

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Memoria presentada por **Ana Piñeiro Moyá** para optar al grado de Doctor en Ciencias Biológicas por la Universidad Autónoma de Madrid

Bajo la dirección de la **Dra. Isabel Barja Núñez**, Profesora del Departamento de Biología de la Universidad Autónoma de Madrid

El doctorando

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A mis padres
A mi hermano
A mis abuelos

Una tesis for you

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INTRODUCCIÓN GENERAL

Generalidades y distribución del gato montés

La familia *Felidae* cuenta en la Península Ibérica con dos únicos representantes, el lince ibérico (*Lynx pardina*) y el gato montés (Aymerich 1982). El gato montés europeo es un carnívoro de tamaño medio, con una morfología similar a la de la forma atigrada del gato doméstico, pero del que se diferencia por su mayor tamaño corporal y por presentar menos rayas en su pelaje. Su pelaje es de color gris o de tonos pardos con un patrón bien definido de rayas negras en el cuerpo y en las patas. Uno de los principales rasgos en el pelaje que lo caracteriza son las 4 líneas oscuras que van desde las orejas hasta la frente convergiendo en una línea longitudinal que recorre todo el lomo (Kitchener 1995, Nowell y Jackson 1996). Su cabeza es robusta, ancha, con orejas pequeñas, hocico corto y rosado, bigotes largos y ojos de color ambarino con pupila vertical. La cola es más ancha y larga que en la forma doméstica y presenta de 2 a 5 anillos negros, terminando siempre en una borla redonda y negra (Kitchener 1995, Daniels et al. 1998). A pesar de que estas características parecen claramente distintas entre gatos monteses y domésticos y de haberse publicado varios trabajos en los cuales se ha comparado la morfología externa de los gatos monteses, los gatos domésticos y sus híbridos (Hubbard et al. 1992, Daniels et al. 2001), su diferenciación en condiciones naturales sigue resultando compleja. Sin embargo, las técnicas moleculares desarrolladas en los últimos años están siendo de gran ayuda para realizar su identificación en muestras de tejido, sangre, pelo y heces (Randi et al. 2001, Lecis et al. 2006, Oliveira et al. 2008a, Oliveira et al. 2010).

La distribución de las especies responde a procesos ecológicos que operan bajo una serie de factores, como son los requerimientos individuales de la especie o los factores ambientales (Guisan y Zimmermann 2000). La distribución del gato montés es una de las más amplias dentro de los felinos, incluyendo Europa occidental, la mayor parte de África y regiones de Arabia y Asia (Nowell y Jackson 1996, Sunquist y Sunquist 2002) (Fig. 1). Sin embargo, su distribución actual está muy fragmentada, con poblaciones aisladas en países como Escocia, Alemania o Bélgica (Stahl y Artois 1991), donde la especie ha quedado relegada a las zonas montañosas más agrestes para huir de las perturbaciones humanas (Stahl y Artois 1994). En la Península Ibérica está presente en la mayor parte del territorio, aunque su distribución es discontinua (Fig. 2). En un estudio reciente realizado sobre los requerimientos

que explican la distribución del felino en España, se observó que los factores que determinan su presencia son el rango altitudinal y la abundancia de las presas (Ferreira et al. 2010).

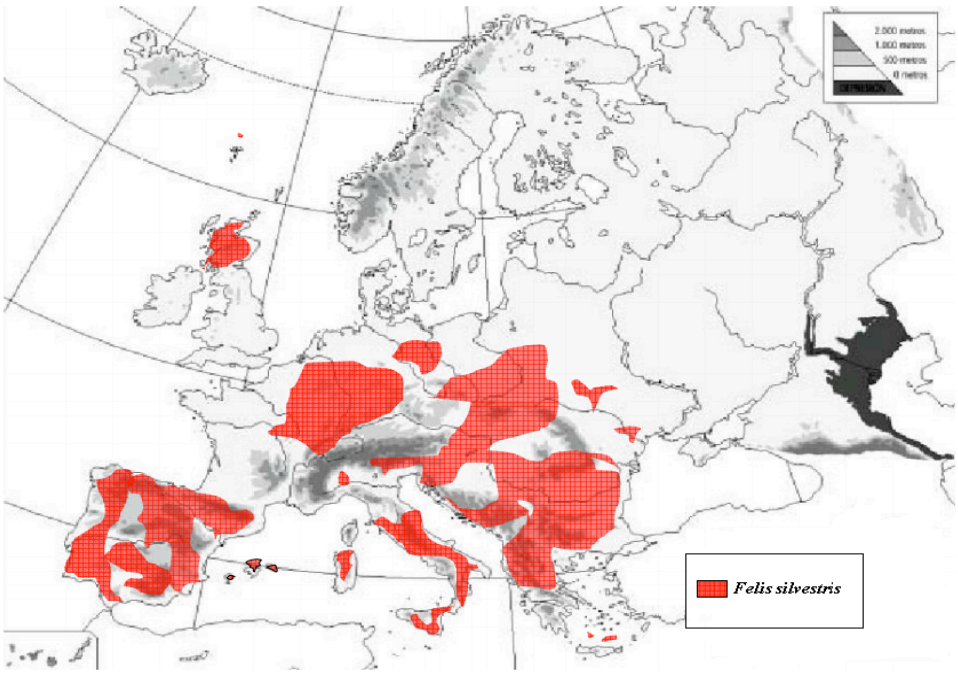


Fig. 1. Distribución del gato montés en Europa (modificada de Mitchell-Jones et al. 1999).

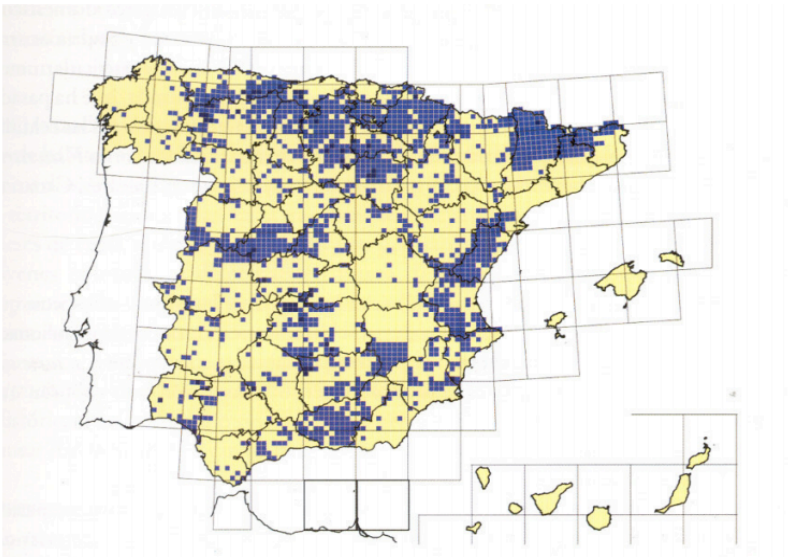


Fig. 2. Distribución del gato montés en España (Palomo y Gisbert 2002).

Uso del espacio y del hábitat y relaciones de competencia

La ecología animal estudia la relación de las especies con su entorno, principalmente el tipo de alimentos que consumen y la variedad de hábitats que ocupan (Johnson 1980). La alteración y fragmentación del hábitat constituyen una de las principales amenazas para la mayoría de los mamíferos. Por ello, es necesario conocer la superficie que necesita cada individuo para poder sobrevivir y perpetuarse con el fin de aplicar herramientas adecuadas de gestión del medio en el que habitan las especies silvestres.

El gato montés es un animal solitario, excepto en la época reproductora, y territorial (Corbett 1979, Stahl 1986). El felino presenta solapamiento intersexual, de modo que el territorio de un macho abarca el de varias hembras (Corbett 1979). El tamaño de los territorios en Europa varía considerablemente entre las distintas zonas de su área de distribución. En Escocia, estudios realizados sobre el uso del espacio con ejemplares radiomarcados han determinado territorios de 175 ha para machos y 459 ha para hembras (Daniels et al. 2001). En Europa continental el territorio de la especie es mucho mayor para ambos sexos (Francia: machos 573 ha y hembras 184 ha [Stahl et al. 1988]; Suiza: machos 2.292 ha y hembras 369 ha [Liberek 1999]; Eslovenia: machos 895-1.876 ha y hembras 264-1.275 ha [Potonick et al. 2005]). El territorio de un macho de gato montés en la Península Ibérica oscila entre 430 y 5.017 ha, mientras que el territorio de una hembra varía entre 87 y 667 ha (Urra 2003, Ferreira 2010).

En ecología se conoce como hábitat el lugar físico donde los individuos de una especie pueden sobrevivir y reproducirse, y es resultado de la combinación de factores bióticos y factores abióticos (alimento, cobertura vegetal, agua, presencia de predadores o competidores, temperatura y precipitación) (Morrison et al. 2006). La selección de hábitat es definida como el uso que hace un animal de un tipo determinado de hábitat en comparación con todos los hábitats disponibles en el medio en el que vive (Johnson 1980, Thomas y Taylor 1990) y es uno de los aspectos más estudiados, junto con la ecología trófica, en todo el rango de distribución del gato montés en Europa (Escocia: Easterbee et al. 1991, Daniels et al. 2001; Francia: Stahl et al. 1988; Suiza: Liberek 1999; España: Lozano et al. 2003, Barja y Bárcena 2005, Lozano 2010; Eslovenia: Potocnik et al. 2005; Portugal: Sarmento et al. 2006, Monterroso et al. 2009; Alemania: Klar et al. 2008, Jerosch et al. 2010). La abundancia de

estudios se debe probablemente a que la alteración del hábitat es una de las principales amenazas y de las más graves a la que está sometido el felino (IUCN 2011). Las preferencias de hábitat para el gato montés en Europa varían entre las diferentes regiones bioclimáticas y la disponibilidad de sus presas principales parece explicar estas diferencias (Easterbee et al. 1991, Urra 2003, Lozano et al. 2007, Monterroso et al. 2009). Así, en la región bioclimática atlántica el hábitat típico del gato montés son los bosques mixtos de frondosas con un sotobosque bien desarrollado (Ragni 1981, Schauenberg 1981, Stahl y Leger 1992, Sarmento et al. 2006), lo que guarda relación con la abundancia de roedores que proporciona este tipo hábitat (Gosálbez y López-Fuster, hUallacháin y Madden 2011). El bosque caducifolio le proporciona, además, por la presencia de árboles viejos y muertos, una gran cobertura para poder cazar al acecho, zonas de refugio y madrigueras (Stahl y Artois 1991, Klar et al. 2008). Asimismo, el gato montés selecciona positivamente áreas rocosas (Klar et al. 2008, Monterroso et al. 2009) y zonas ribereñas (Daniels et al. 2001, Lozano et al. 2003, Barja y Bárcena 2005, Klar et al. 2008, Jerosch et al. 2010). Sin embargo, la especie rechaza los bosques maduros de coníferas (Corbett 1979), lo que puede estar relacionado con una menor abundancia de roedores (Easterbee et al. 1991) y una mayor persistencia de la nieve (Dötterer y Bernhart 1996), ya que el felino evita las zonas nevadas (Liberek 1999) debido a una mayor dificultad para cazar (Corbett 1979). Por otro lado, en la región bioclimática mediterránea el gato montés selecciona las zonas de matorral, debido a que este hábitat le proporciona refugio (Corbett 1979, Lozano et al. 2003, Thiel 2005, Monterroso et al. 2009) y una gran riqueza de presas (Lozano et al. 2003, Ballesteros-Duperón et al. 2005). Asimismo, el felino muestra una selección positiva hacia zonas mixtas de pastizal y matorral (Lozano et al. 2003, Lozano 2010) y zonas de ecotono entre áreas con matorral y sin él (Ragni 1978). Por último, destacar que el felino también utiliza zonas agrícolas y pastizales (Easterbee et al. 1991, Nowell y Jackson 1996, Lozaro et al. 2003), principalmente durante la noche para cazar (Ragni 1978, Potocnik et al. 2005, Thiel 2005), evitando estos hábitats durante el día debido a las perturbaciones humanas (Monterroso et al. 2009).

En un mismo hábitat pueden coexistir especies de carnívoros que aprovechan recursos similares (especies simpátricas). La competencia entre dos especies es más probable cuando se superponen sus áreas de distribución geográfica y muestran un tamaño corporal y hábitos alimenticios similares (Barrientos y Virgós 2006). No obstante, existen una serie de

mecanismos que favorecen la coexistencia de especies simpátricas con requerimientos similares (MacArthur y Levins 1967). Estos mecanismos incluyen la segregación trófica, el uso de hábitats diferentes o una segregación temporal (Schoener 1974), aunque, según el *principio de exclusión* de Gause (1934) dos especies con idénticas exigencias ecológicas no pueden coexistir y una de ellas termina compitiendo con la otra. La diferencia de tamaño corporal parece ser el principal factor implicado en la aparición de interacciones negativas entre competidores (Donadio y Buskirk 2006), pudiendo los individuos de mayor tamaño depredar sobre los más pequeños (Palomares y Caro 1999). La mayoría de los estudios realizados sobre las relaciones de competencia del gato montés con otros carnívoros han examinado la competencia trófica (Aymerich et al. 1982, Carvalho y Gomes 2004, Biró et al. 2005, Barrientos y Virgós 2006). Sin embargo, los estudios de competencia por el hábitat con otros carnívoros son escasos (Mangas et al. 2008), basándose la mayoría de ellos en la relación del gato montés con sus formas domésticas e híbridas (Corbett 1979, Biró et al. 2004, Germain et al. 2008). No obstante, para establecer una estrategia eficaz de conservación del gato montés es fundamental conocer cómo le afecta la presencia en el medio de especies de carnívoros competidoras, principalmente aquellas con las que comparte el mismo nicho trófico.

Es importante conocer la relación entre la fauna silvestre y el medio en el que se desenvuelven las especies para identificar las áreas y recursos que influyen en la supervivencia de las poblaciones naturales. Los resultados aportados por estudios científicos sobre el uso y selección del hábitat de las especies en libertad son una herramienta imprescindible para establecer estrategias de conservación más eficaces, tanto de la fauna como del medio, especialmente para aquellas especies esquivas y solitarias, como es el gato montés.

Ecología trófica

La ecología trófica es una rama de la ecología que se encarga de estudiar la relación entre los distintos niveles tróficos que integran un ecosistema, incluyendo la interacción depredador-presa y la competencia entre especies por los recursos tróficos (Hairston et al. 1960). En carnívoros, en la mayoría de los estudios publicados sobre ecología trófica se analiza su dieta, siendo éste el aspecto mejor conocido de su biología en casi todas las especies, incluyendo el gato montés.

En el área de distribución del gato montés los roedores y el conejo (*Oryctolagus cuniculus*) constituyen su presa principal. En Europa los conejos son su presa principal en algunas zonas (Corbett 1979, Schauenberg 1981). Sin embargo, en la mayoría de los estudios europeos se señala a los roedores como la especie presa más consumida, principalmente los murinos y los microtinos (Lindemann 1953, Condé et al. 1972, Nasilov 1972, Ragni 1978, Kožena 1990, Liberek 1999, Carvalho y Gomes 2004, Biró et al. 2005), y los conejos aparecen en la dieta de manera ocasional como presa secundaria (Sládek 1973, Fernandes 1993, Sarmento 1996).

Este patrón de variación geográfica observado en Europa en la dieta del gato montés parece estar relacionado con la región biogeográfica en la que se haya realizado el estudio (Lozano et al. 2006). En España, la región mediterránea ocupa el centro y sur peninsular, mientras que la región atlántica se sitúa en el norte peninsular, presentando estas dos regiones hábitats y presas bien distintos. Así, la región mediterránea está dominada por vegetación esclerófita y la base de la dieta del gato montés son los conejos (Gil-Sánchez et al. 1999, Malo et al. 2004, Monterroso et al. 2009), mientras que en la región atlántica, en la que predominan los bosques mixtos, el felino consume principalmente roedores (Aymerich et al. 1980, Aymerich 1982, Urra 2003). También se ha observado un patrón biogeográfico en el consumo de roedores por el gato montés en relación con la latitud. El gato consume principalmente murinos en zonas situadas a bajas latitudes y microtinos en latitudes altas (Lozano et al. 2006). La presencia en la dieta del gato montés de otras presas como reptiles, aves, insectos y mamíferos como el corzo y la liebre, está ampliamente documentada, tanto en la Península Ibérica (Aymerich et al. 1980, Sarmento 1996, Gil-Sánchez et al. 1999, Moleón y Gil-Sánchez 2003, Carvalho y Gomes 2004, Malo et al. 2004) como en el resto de Europa (Condé et al. 1972, Ragni 1978, Corbett 1979, Kožená 1990, Biró et al. 2005).

La especialización y el generalismo son definidos en términos de utilización de los recursos (alimento y hábitat). Por lo que concierne a la alimentación, una especie es considerada *especialista trófica* cuando está adaptada a un tipo de presa, y su preferencia hacia ella es casi constante, independientemente de su disponibilidad o vulnerabilidad (Holling 1959, Glasser 1982). Por el contrario, una especie es considerada *generalista* u *oportunista trófica* cuando tiene un amplio espectro trófico y es capaz de explotar los alimentos que están más disponibles en el medio en cada estación y zona, variando su dieta en función de la

disponibilidad y/o facilidad de captura de las distintas especies presa. El depredador generalista, según la *hipótesis de la presa alternativa*, cuando disminuye la abundancia de un tipo de presa, comienza a consumir la especie presa más abundante y/o accesible respecto a la primera (Angelstam et al. 1984). Sin embargo, con frecuencia las estrategias tróficas de los carnívoros se encuentran a medio camino entre el especialismo y el generalismo tróficos, y éstos son clasificados como *especialistas facultativos* cuando se especializan en el consumo de una especie presa pero pueden cambiar su consumo hacia otra presa alternativa cuando ésta última es más rentable en términos energéticos (Glasser 1982).

Según el *modelo de selección de dieta* propuesto por Ellis et al. (1976), la selección de una especie presa está influenciada por una serie de factores, entre los que se encuentran la disponibilidad de alimento, los requerimientos energéticos del animal, la preferencia y la selectividad. En este modelo la disponibilidad de alimento es definida como una medida de la abundancia en el medio de los distintos tipos de recursos tróficos accesibles para el consumidor. La preferencia es un reflejo de la predilección del animal por un determinado tipo de alimento cuando en el medio todos los recursos tróficos están disponibles en la misma proporción (Ellis et al. 1976). La selectividad es el proceso en el cual un animal elige un alimento determinado, independientemente de la abundancia de los diferentes recursos tróficos que tiene a su alcance (Ellis et al. 1976). Por tanto, el estudio de la selectividad trófica de una especie debe basarse en la comparación del consumo y de la disponibilidad de los alimentos (Johnson 1980).

A pesar de la importancia de los factores arriba mencionados para avanzar en el conocimiento de las estrategias tróficas que siguen las distintas especies de carnívoros, la mayoría de los trabajos realizados son estudios meramente descriptivos de la dieta y no tienen en cuenta los factores mencionados por Ellis et al. (1976). A este respecto, hay que destacar un estudio científico realizado en el sur de la Península Ibérica con gato montés (Moleón y Gil-Sánchez 2003) en el que se ha tenido en cuenta la disponibilidad anual de la presa principal, el conejo, para relacionarla con su consumo. En ese estudio los autores ponen en duda la condición del gato montés como especialista trófico y lo definen como un especialista facultativo, por seleccionar el conejo como especie presa cuando éste se encontraba en altas densidades y alimentarse de roedores cuando el conejo se volvía escaso o no estaba presente.

No obstante, cabe destacar que en este estudio los autores no tuvieron en cuenta como variaba la vulnerabilidad de las especies presa a lo largo del año.

Por último, hay que señalar que en la actualidad no existe ningún trabajo científico, excepto el incorporado en la presente tesis doctoral, en el que se tuviera en cuenta la vulnerabilidad de captura de la presa principal para valorar la estrategia trófica del gato montés, a pesar de ser, junto con la disponibilidad de la presa, piezas claves a la hora de definir la estrategia trófica de cualquier especie.

Comunicación química

Los animales, tanto sociales como solitarios, interactúan entre ellos y por ello las señales comunicativas resultan indispensables en sus vidas. La comunicación animal puede definirse como la transmisión de información de un individuo (emisor) a otro (receptor), influyendo dicha información sobre el comportamiento de este último mediante señales que han evolucionado para este fin (Wilson 1975). Las señales comunicativas en carnívoros se pueden agrupar en auditivas, táctiles, visuales y químicas (Eisenberg y Kleiman 1972). La comunicación química en carnívoros se realiza a través de señales olorosas procedentes de secreciones glandulares, orina y heces (Gorman y Trowbridge 1989). El conjunto de comportamientos usados para depositar estas señales olorosas sobre distintos elementos del medio o sobre otros individuos se conoce con el nombre de marcaje oloroso (Johnson 1973, Agosta 1992). El canal químico presenta varias ventajas frente a los otros canales, así, la transmisión del mensaje tiene lugar en ausencia del emisor y se necesita una pequeña cantidad de compuestos semioquímicos para dejar una señal de larga duración (Eisenberg y Kleiman 1972, Gosling y Roberts 2001). También, las señales olorosas son eficaces donde otras señales son difíciles de detectar, como en zonas de vegetación densa, bajo el suelo o en períodos de oscuridad (Gorman y Trowbridge 1989).

El gato montés, como la mayoría de los felinos, es un animal nocturno, territorial y solitario la mayor parte del año, excepto durante la reproducción (Kitchener 1995, Sunquist y Sunquist 2002). Por ello, su principal forma de comunicación es a través de señales químicas contenidas en la orina, secreciones procedentes de glándulas especializadas (interdigitales, anales y faciales) y heces. Estas marcas olorosas son depositadas sobre sustratos destacados del

medio (rocas, plantas, troncos caídos, etc.). El uso de sustratos vegetales como postes de marcaje en felinos está ampliamente documentado (guepardo, *Acinonyx jubatus*: Eaton 1970a; gato montés: Corbett 1979; tigre, *Panthera tigris*: Smith et al. 1989; leopardo, *Panthera pardus*: Bothma y le Riche 1995). Sin embargo, en mamíferos no existen apenas estudios en los cuáles se determine qué características de las plantas las hacen más atractivas para depositar las señales olorosas (Barja 2009). La producción y el mantenimiento de las señales químicas implican un coste elevado (Gosling y Roberts 2001), por lo que las marcas olorosas no son depositadas al azar en el territorio, si no en sustratos que maximizan su detección por parte de otros individuos (Gosling 1981). De ese modo, muchos carnívoros eligen determinadas zonas y sustratos que aumentan la efectividad visual y olorosa de la señal (Corbett 1979, Vilà et al. 1994, Barja et al. 2004, Tsegaye et al. 2008, Barja 2009) y que amplifican el espacio activo de los componentes químicos (Alberts 1992, Gosling y Roberts 2001).

Las marcas olorosas en felinos desempeñan varias funciones, entre las que se encuentran la defensa del territorio (Eaton 1970a, Smith et al. 1989), detección de la condición reproductiva (Wemmer y Scow 1977, Molteno et al. 1998, Richardson 1998), identificación individual y sexual (Wemmer y Scow 1977) e indicación del estado inmunológico (Zala et al. 2004). En el gato montés la función principal de las marcas olorosas es la defensa del territorio (Stahl y Leger 1992). La defensa activa de los territorios supone un coste elevado en tiempo y energía, por lo que la deposición de las marcas olorosas le permite al animal defender su territorio de un modo pasivo frente a potenciales intrusos (Gosling 1982), pudiendo dedicar más tiempo a otras actividades como la búsqueda de alimento.

Los animales compiten por los recursos, tales como alimento, refugio o pareja (Maher y Lott 1994, Gese 2001). Un modo de defender estos recursos es a través de la deposición de marcas olorosas (Ralls 1971), las cuales actúan como una defensa pasiva de esos recursos, evitando encuentros agonísticos entre potenciales competidores (Gosling y Mackay 1990, Sillero-Zubiri y Macdonald 1998, Zub et al. 2003). Así, los intrusos analizan la información que transmite las señales y evalúan los costes y beneficios de competir con los propietarios por esos recursos (Gosling y MacKay 1990, Gosling y Roberts 2001). Corbett (1979) en un estudio realizado en Escocia ha señalado que el gato montés muestra un comportamiento de marcaje territorial más acusado en las áreas de descanso y en las principales zonas de caza. También

Robinson y Delibes (1988) señalaron que los linces señalan más con heces los cruces de camino que otras zonas de su territorio por ser éstos áreas favorables de caza, donde la probabilidad de encuentros del depredador con las presas es mayor.

Diferentes estudios científicos han indicado que existen diferencias interespecíficas e intraespecíficas en los patrones de marcaje oloroso de las diferentes especies de carnívoros (Macdonald 1980, Mellen 1993). Por tanto, es necesario conocer cómo varían los patrones de marcaje fecal en las diferentes especies de carnívoros y en los distintos hábitats dentro de la misma especie, al ser de gran utilidad estos estudios para establecer en el campo los itinerarios de muestreo a fin de optimizar la recolección de muestras fecales para llevar a cabo estudios moleculares o fisiológicos, entre otros. Los conocimientos del comportamiento de marcaje fecal también son de utilidad en los estudios de distribución y estima de abundancias de las diferentes especies, así como en el control de especies plaga y en biología de la conservación, siendo de gran utilidad en los programas de conservación *ex situ*. Por último, el marcaje oloroso también puede ser utilizado como indicador de bienestar animal en cautividad.

Estado de conservación

El gato montés se encuentra incluido dentro de la categoría "preocupación menor" a nivel europeo (IUCN 2011) y como "especie estrictamente protegida" por el Convenio de Berna (Anexo II). Además, el gato montés está incluido en el Anexo IV de la Directiva Hábitats de la UE y en el Anexo II del Reglamento CITES. A nivel nacional, el gato montés está catalogado como "de interés especial" en el Catálogo Nacional de Especies Amenazadas y "casi amenazado" en el Libro Rojo de los Mamíferos de España (Palomo y Gisbert 2005).

Las poblaciones del gato montés en Europa se vieron reducidas a finales del siglo XIX y principios del siglo XX como consecuencia de la persecución humana directa a través de las campañas de control de predadores, lo que provocó un fuerte declive de sus poblaciones del que la especie parece estar recuperándose (Stahl y Leger 1992, McOrist y Kitchener 1994). Sin embargo, a pesar de que el gato montés se encuentra protegido en Europa, su caza ilegal sigue siendo un problema (McOrist y Kitchener 1994, Virgós y Travaini 2005). Otras amenazas que afectan a la especie en Europa son la alteración del hábitat y la fragmentación de las poblaciones (Easterbee et al. 1991, Stahl y Artois 1991, Kitchener 1995, Nowell y Jackson

1996), los atropellos (Klar et al. 2009) y la reducción de sus presas por interferencia con competidores herbívoros (Lozano et al. 2007). También la hibridación con los gatos domésticos es una amenaza que preocupa a la comunidad científica, no sólo por la pérdida genética de las poblaciones naturales de gato montés, sino por el contagio de enfermedades procedentes de la forma doméstica (Hubbard et al. 1992, McOrist y Kitchener 1994, Pierpaoli et al. 2003, Lecis et al. 2006, Oliveira et al. 2008b). Sin embargo, estudios recientes llevados a a cabo en distintas poblaciones europeas indicaron que la hibridación es un problema local (Randi et al 2001, Pierpaoli et al 2003, Lecis et al 2006, Oliveira et al 2008b), con una tasa de introgresión menor del 4% en España (Ruiz-García et al. 2001).

Respuesta de estrés fisiológico a factores ambientales

A pesar del gran número de estudios científicos publicados sobre los efectos de las alteraciones en el medio sobre la conservación del gato montés, no hay ningún trabajo en el que se hayan evaluado cuáles son las consecuencias de los cambios en el medio sobre la respuesta de estrés fisiológico en esta especie y apenas existen estudios en otras especies de carnívoros en estado natural. Recientemente ha surgido una nueva disciplina científica dentro de la biología que puede ayudar a mejorar la conservación de las especies a través de enfoques endocrinos, es la denominada Fisiología de la Conservación, que se ocupa de estudiar las respuestas fisiológicas de los organismos a las alteraciones humanas en el medio ambiente que pueden causar o contribuir al declive de las poblaciones (Wikelski y Cooke 2006).

El concepto clásico de estrés implica cambios fisiológicos y neurológicos en el organismo cuando éste ha sido expuesto a un conjunto de agentes nocivos (Selye 1960). Actualmente el estrés es definido como el conjunto de cambios fisiológicos, hormonales y conductuales que permiten a un animal hacer frente a un agente estresante (Romero 2004), siendo considerado como agente estresante o estresor cualquier perturbación capaz de alterar la homeostasis de un organismo. Cuando actúa un agente estresante, los animales responden al mismo aumentando la disponibilidad inmediata de energía en determinados órganos y músculos e inhibiendo aquellos procesos fisiológicos que no son necesarios para la supervivencia inmediata (Sapolsky 1994). Al conjunto de estas respuestas endocrinas se les conoce como respuesta fisiológica de estrés, la cual está regulada por el eje Hipotálamo-

Pituitaria-Adrenal (HPA) (Fig. 3). Cuando actúa un estímulo estresante el hipotálamo segrega el factor liberador de corticotropina (CRF) que estimula a la pituitaria para que libere la adrenocorticotropina (ACTH), la cual circulará por el torrente sanguíneo hasta las glándulas suprarrenales donde activará la secreción de glucocorticoides (Fig. 3).

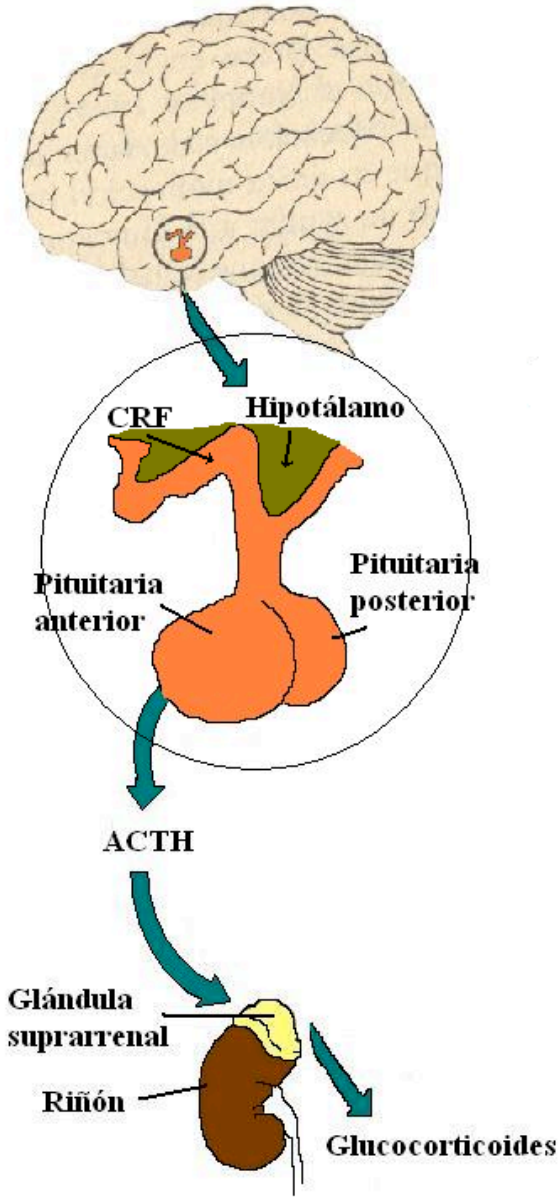


Fig. 3. Esquema de la respuesta fisiológica de estrés en mamíferos (modificado de Sapolsky 1994).

Los glucocorticoides, junto con el glucagón y el sistema nervioso simpático, son los responsables de elevar el nivel de glucosa en sangre y, por tanto, de aportar la energía necesaria al organismo para poder hacer frente al agente estresante (Sapolsky 1994, Wingfield y Romero 2001). Una vez superada la situación estresante, los propios glucocorticoides restauran la homeostasis mediante feedback negativo al disminuir la secreción de CRF y ACTH. Cuando la secreción de glucocorticoides ocurre durante un período corto de tiempo, dicha respuesta es adaptativa y beneficiosa para el organismo. Sin embargo, si la respuesta es a largo plazo, ésta se vuelve tan nociva como el propio agente estresante y da lugar a patologías como supresión reproductora, supresión inmunológica y aparición de úlceras, entre otras (Stewart 2003). Esto reduce la supervivencia y el éxito reproductivo del organismo y, sin duda, la eficacia biológica de las especies y poblaciones (Romero 2004). En la actualidad, los datos acerca de los efectos que tienen las elevaciones de glucocorticoides sobre la eficacia biológica de las especies en su medio natural son limitados. Los estudios de laboratorio sugieren que los efectos sobre la eficacia biológica incrementan a medida que el agente estresante es más severo o más prolongado (Blanchard et al. 1995).

Las concentraciones de glucocorticoides (cortisol, corticosterona y cortisona) han sido usadas en muchos estudios como indicadores fisiológicos de estrés animal (Möstl y Palme 2002). En la década pasada se han desarrollado métodos no invasivos para estudiar las respuestas de estrés fisiológico mediante la cuantificación de los niveles de glucocorticoides en heces. Esta nueva técnica ha sido utilizada en diferentes especies de carnívoros (Young et al. 2004), como licaón (*Lycaon pictus*) (Monfort et al. 1998), hiena manchada (*Crocuta crocuta*) (Goymann et al. 1999), oso grizzly (*Ursus arctos horribilis*) (Von der Ohe et al. 2004), lobo (*Canis lupus*) (Sands y Creel 2004, Barja et al. 2008) y marta europea (*Martes martes*) (Barja et al. 2007, 2011).

La pérdida de biodiversidad que está ocurriendo en las últimas décadas tiene su origen en fenómenos ligados a las actividades humanas, las cuales producen fragmentación, pérdida y transformación del hábitat provocando un gran impacto sobre el medio ambiente (Parmesan 2006, Shepherd y Whittington 2006, Theuerkauf et al. 2007). El efecto de las perturbaciones humanas sobre las poblaciones animales ha sido un tema ampliamente debatido por los distintos sectores implicados (biólogos, gestores de fauna, políticos y público en general). Sin embargo, a pesar de que muchas especies animales se encuentran amenazadas debido en mayor

o menor grado a causas relacionadas con las actividades humanas (Saunders et al. 1991), la mayoría de los estudios realizados analizaron únicamente sus efectos sobre el comportamiento y muy pocos han indagado en el efecto de las mismas sobre las respuestas de estrés fisiológico. No obstante, unos pocos estudios han puesto de manifiesto que para los carnívoros silvestres las actuaciones humanas en el medio pueden constituir agentes estresantes (actividades deportivas en lobo: Creel et al. 2002, presión turística en marta: Barja et al. 2007). En felinos también se ha constatado que las perturbaciones humanas les afectan negativamente, sin embargo, en estos trabajos sólo se ha evaluado la respuesta comportamental (Van Dyke et al. 1986, Gagliuso 1992, Kerley et al. 2002, Ngoprasert et al. 2007) y sólo en unos pocos llevados a cabo en cautividad se evaluó la respuesta de estrés fisiológico en relación con el número de visitantes en las instalaciones (Terio et al. 2004, Montanha et al. 2009). Además de las actividades humanas, otros factores como la calidad del hábitat, el estatus social, la competencia intra e interespecífica y los ritmos fisiológicos circanuales (como por ejemplo la reproducción), entre otros, se ha constatado que también afectan a las concentraciones de glucocorticoides, siendo fuentes potenciales de estrés (Harlow et al. 1990, Barja et al. 2008).

El impacto de los cambios en el medio en el que viven los animales tiene que ver con aspectos fisiológicos que determinan directamente la supervivencia del individuo y su capacidad reproductora. Entre estos aspectos fisiológicos se puede mencionar el estrés, la condición energética y los niveles de inmunocompetencia (McNab 2002). La escasez de alimento puede provocar en los animales diversos cambios fisiológicos, lo que sin duda puede reducir su metabolismo basal, afectando a su capacidad reproductora, dado que la reproducción sólo es posible cuando las reservas grasas del individuo exceden un nivel mínimo (Lochmiller y Deerenberg 2000). La falta de alimento también puede provocar una respuesta de estrés en los animales (Ellis et al. 2012). El estrés nutricional ha sido definido como un estado comportamental y fisiológico que es provocado por la baja calidad y cantidad de alimento disponible para un animal (Trites y Donnelly 2003). Por tanto, el aumento en los niveles de estrés puede ser debido a estresores nutricionales como una baja calidad del alimento (Taillon y Côté 2008) y/o a una escasa abundancia del mismo (Foley et al. 2001).

El aumento de las hormonas glucocorticoides también tiene efectos deletéreos en el sistema inmunológico, haciendo al individuo más vulnerable a la colonización por agentes patógenos y comprometiendo así su supervivencia y capacidad reproductora (Lochmiller y

Deerenberg 2000). En la mayoría de las especies los parásitos no tienen efectos negativos sobre el hospedador y ambos viven en armonía. Sin embargo, bajo determinadas condiciones ambientales (baja calidad de hábitat) y en individuos con deficiente condición física (altos niveles de estrés, función inmune disminuida, desnutrición, etc.) la infección por agentes patógenos en animales silvestres puede diezmar sus poblaciones (Feldhamer et al. 1999). En relación con esto, algunos estudios indicaron que la interacción glucocorticoides-parásito es a menudo bidireccional. El aumento en los niveles de estrés fisiológico puede deprimir el sistema inmune (inmunosupresión inducida por estrés), pero también la infección parasitaria puede ser la responsable del aumento en los niveles de estrés (estrés inducido por infección) (Brown y Fuller 2006). También, hay que destacar que no sólo el aumento en la respuesta de estrés influye en el sistema inmune, pues también la calidad de hábitat (reducción en la cantidad y calidad del alimento) puede reducir la capacidad inmune y aumentar los niveles de glucocorticoides. La calidad del hábitat no sólo afecta a los niveles de glucocorticoides si no también a la eficacia biológica de los individuos (Huey 1991).

Los espacios naturales protegidos, creados principalmente para conservar la biodiversidad y la fauna silvestre, ofrecen una enorme oportunidad para el turismo de naturaleza (Redd y Merenlender 2008). Así, en las últimas décadas el turismo en áreas protegidas ha aumentado considerablemente y se espera que lo haga aún más en un futuro próximo (Redd y Merenlender 2008, Balmford et al. 2009). Este aumento va a reportar importantes beneficios económicos para los espacios protegidos, pero puede tener efectos negativos sobre la fauna, entrando en conflicto las actividades turísticas con la conservación de las especies, al actuar las actividades de recreo como potenciales estresores. Por tanto, los estudios fisiológicos pueden ser claves para comprender la respuesta de las especies a los nuevos retos ambientales y para que las estrategias de conservación tengan éxito.

ÁREA DE ESTUDIO

El área de estudio incluye el Parque Natural Os Montes do Invernadeiro. El Parque ocupa una extensión de 5.722 ha y se encuentra situado al noroeste de la Península Ibérica (Fig.4). Este espacio natural de carácter montañoso forma parte del gran Macizo Central Ourensano y limita por el norte con los montes comunales de Camba (Laza) y Vilariño de Conso, y con la Sierra de Queixa (Chandrexa de Queixa); al sur con el embalse de As Portas y el monte comunal de Campobecerros (Castrelo do Val); al este con la divisoria de los ríos Ribeira Grande y Conso; y al oeste con la Sierra de O Fial das Corzas (Laza). Su altitud oscila entre los 830 y los 1.707 m.s.n.m. que tiene el punto más alto, O Seixo.

Los Montes do Invernadeiro presentan un clima mediterráneo con influencias atlánticas, lo que se hace patente en la vegetación que los conforman. A lo largo del año existen variaciones muy marcadas en las temperaturas, siendo la temperatura media de 3,1º C en diciembre y enero y de 17,2º C en julio y agosto para el periodo de recolección de los datos de la tesis doctoral. Las precipitaciones son abundantes, con una media anual de 1.245 L/m^2, y gran parte de ellas en forma de nieve, principalmente durante los meses de diciembre y enero (datos provenientes de la estación meterológica que tiene situada la Xunta de Galicia en el parque natural).

El área de estudio se encuentra localizada en una zona de transición entre las regiones biogeográficas Eurosiberiana y Mediterránea, lo que se manifiesta en la alternancia de bosques relictos atlánticos y algunas especies de ambientes mediterráneos (Castroviejo 1977, Pulgar 2004) (Fig. 4). La orografía de la zona es consecuencia del glaciarismo que modeló estas alturas en el pasado dando como resultado una sucesión de profundos valles y altas cumbres desde las que se puede observar un circo glaciar y la caída de varias cascadas en algunas zonas. El parque natural está constituido por dos valles que forman una V que recorren toda el área de estudio, el formado por el río Ribeira Grande al norte y el formado por el Ribeira Pequena al sur (Fig. 4).

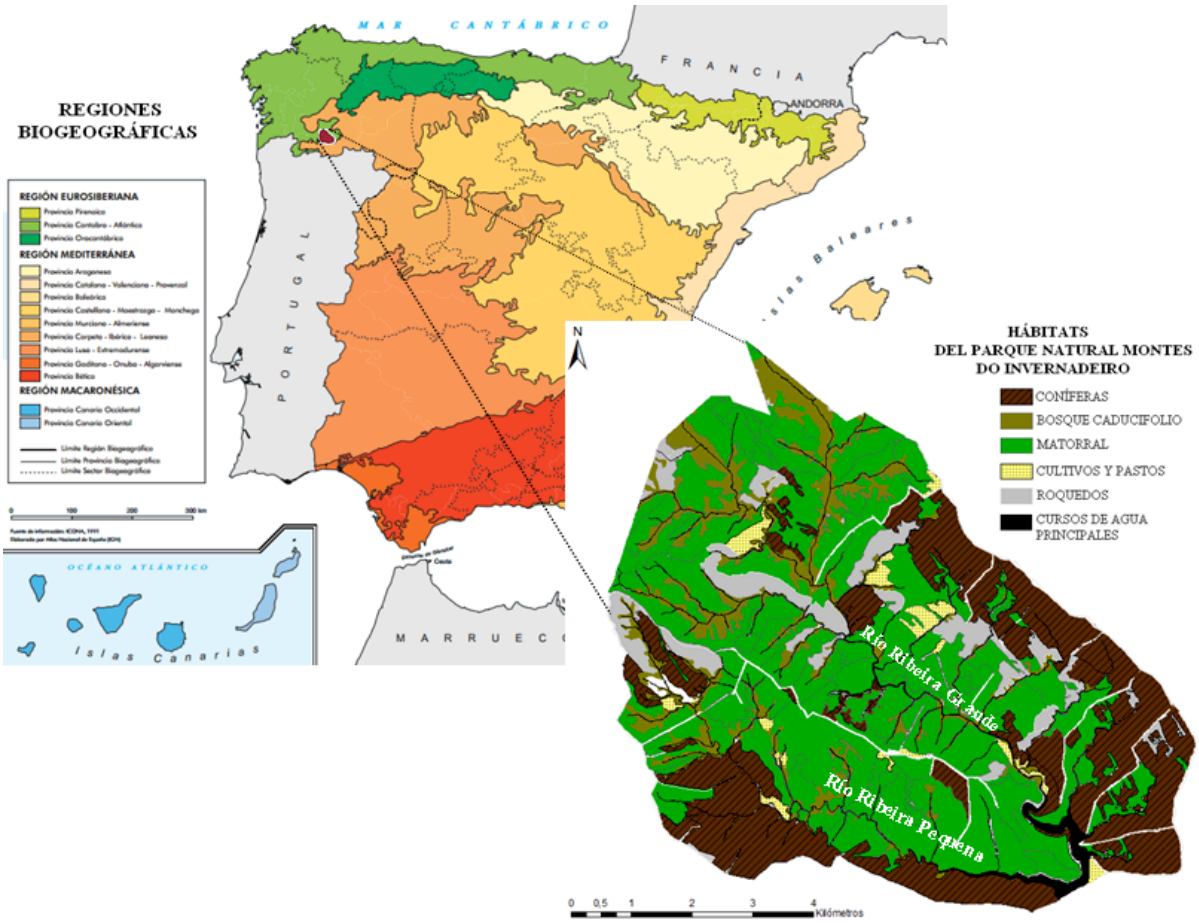


Fig. 4. Situación geográfica del Parque Natural Os Montes do Invernadeiro en la Península Ibérica y los distintos tipos de hábitat presentes en el mismo.

La comunidad vegetal predominante en todo el Parque Natural es el brezal y está formado principalmente por asociaciones de brezo rubio (*Erica australis),* carquesia (*Pterospartum tridentatum*) y jaguarzo (*Halimium lasianthum*). En las zonas más húmedas del parque está presente el brezo blanco (*Erica arborea*). Entre las comunidades de matorral están también presentes los piornales y retamales, con especies como la retama blanca (*Genista florida*) y la escoba blanca (*Cytisus multiflorus*). En las cotas más altas predominan la quiruela (*Erica umbellata*) y las praderas con arándanos (*Vaccinium myrtillus*). La influencia mediterránea se pone de manifiesto por la presencia del roble melojo (*Quercus pynenaica*) y

del tejo (*Taxus baccata*) que aparece de forma ocasional. En los valles y vaguadas del parque están presentes bosques relictos atlánticos, formados por asociaciones de roble (*Quercus robur*), abedul (*Betula celtiberica*) y acebo (*Ilex aquifolium*). Amplias extensiones están cubiertas por bosques de repoblación de pino albar (*Pinus sylvestris*) (Fig. 4 y Fig. 5).

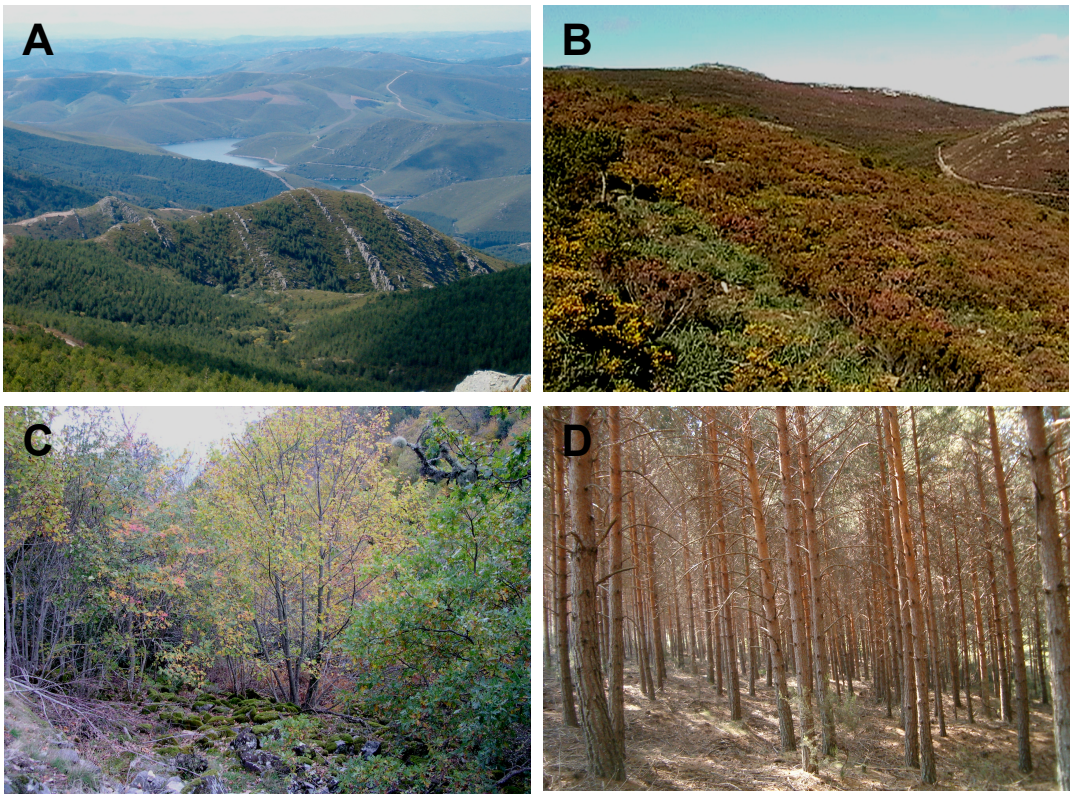


Fig. 5. Diferentes tipos de hábitats predominantes del área de estudio (Parque Natural Os Montes do Invernadeiro). A.− Vista general desde las zonas de cumbre, donde se divisa el valle Ribeira Pequena y el embalse. B.− Matorral, el hábitat principal del área de estudio. C.− Bosque caducifolio asociado a los cursos de agua de las vaguadas y valles. D.− Bosque de repoblación de pino albar.

El parque natural se divide en tres zonas según el grado de protección (Fig. 6): 1) *Zona de uso público restringido* (2.466 ha); se trata de una zona que puede ser visitada por todas las personas que acceden al parque. Desde el valle de la Ribeira Pequena al valle de la Ribeira Grande el recorrido se realiza en coche y el resto de las rutas se hacen a pie (ver fig. 6). Dentro de esta zona está ubicada el aula de naturaleza, así como varias casas donde se alojan

investigadores y guardería cuando es necesario. 2) *Zona de protección especial* (2.142 ha); se trata de una zona en la que los grupos de visitantes (principalmente alumnos de colegios y Universidades) sólo pueden desplazarse a pie y siempre acompañados por los biólogos de la Xunta de Galicia para realizar actividades de educación ambiental. 3) *Zona de reserva integral* (1.114 ha); sólo se le permite el acceso en coche y a pie a investigadores y al personal del parque cuando es necesario. Para los visitantes está prohibido el acceso en cualquier época del año. Las zonas están bien definidas por carteles informativos y barreras para impedir el paso de vehículos a aquellas zonas donde no está permitido el acceso. Las restricciones de acceso para los visitantes en cada zona son las mismas durante todo el año.

Dentro del área protegida no existe población humana asentada. Sin embargo, la Xunta de Galicia adecuó dentro del valle Ribeira Grande unas aulas de naturaleza equipadas para que los grupos de estudiantes puedan desarrollar actividades de educación ambiental, pudiendo pernoctar dentro del parque un máximo de tres noches, con previo permiso del Servicio de Conservación de la Naturaleza de Ourense dependiente de la Xunta de Galicia. El número de grupos que visita el espacio natural protegido es mayor durante los meses de primavera y verano según los datos facilitados por este servicio. Además de los grupos, pueden acceder a la zona de uso público restringido otras personas interesadas en visitarlo, las cuales debe también solicitar una autorización previa al servicio mencionado, pero no pueden pernoctar en el mismo. Este tipo de visitantes sólo pueden usar los vehículos para llegar al valle de la Ribeira Grande y luego hacer recorridos a pie por las rutas señalizadas en los carteles informativos, no pudiendo salir de las mismas. Todas estas rutas discurren por la zona de uso público restringido (Fig. 6). El número máximo de visitantes diarios es de 88 personas los días laborables y de 108 los fines de semana y días festivos.

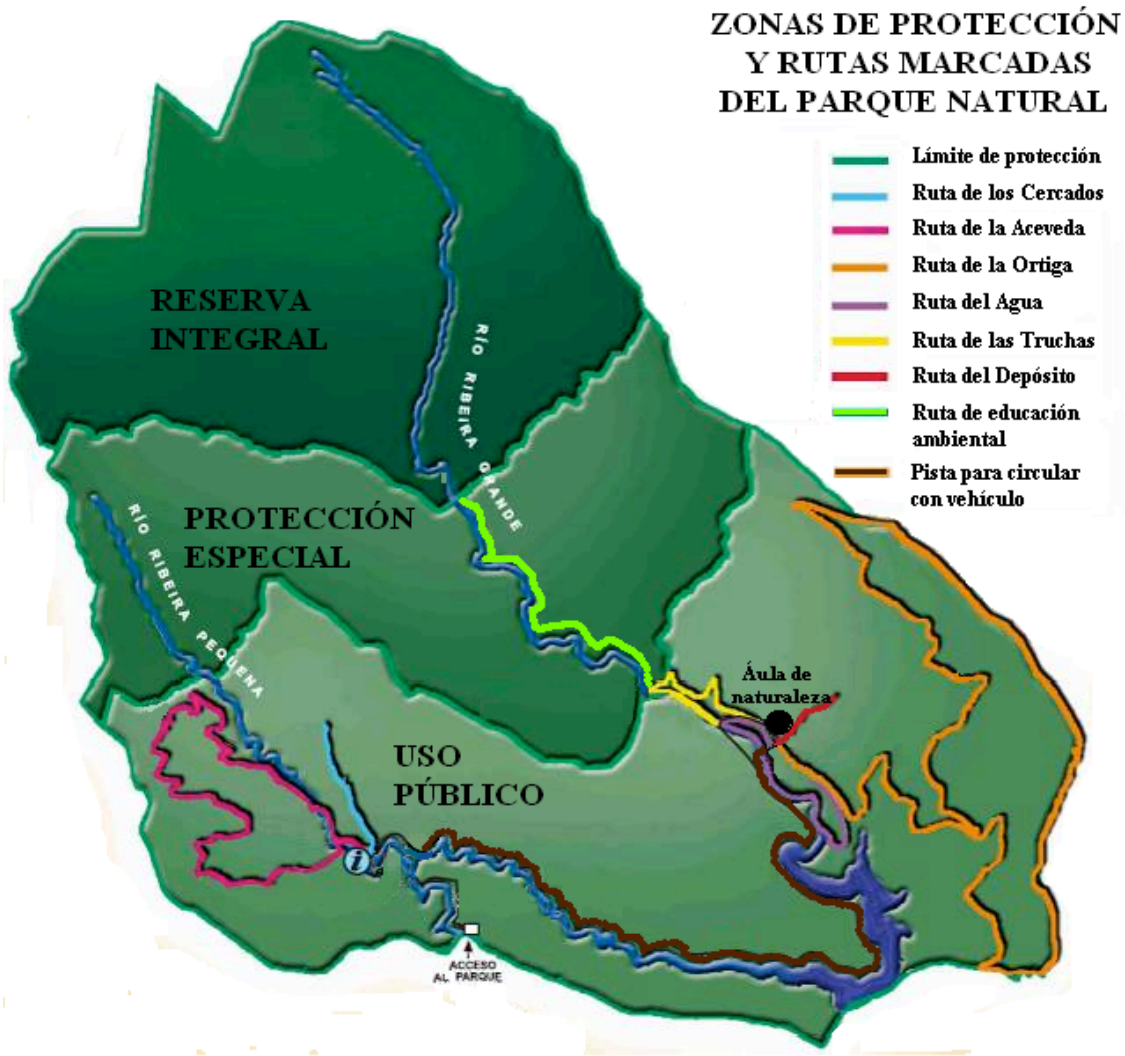


Fig. 6. Plano general del área de estudio con las tres zonas de protección y los recorridos a pie establecidos por la Xunta de Galicia para los visitantes que sólo pueden acceder a la zona de uso público restringido.

El parque natural Os Montes do Invernadeiro cuenta con una gran diversidad biológica, 150 especies de vertebrados, de las cuales 90 son aves, entre las que hay que destacar la presencia del águila real (*Aquila chrysaetos*), el halcón peregrino (*Falco peregrinus*), el águila culebrera (*Circaetus gallicus*), el aguilucho cenizo (*Circus pygargus*) y el abejero europeo (*Pernis apivorum*). Asimismo, la zona está ocupada por una importante población de carnívoros, entre otros, destaca la presencia del lobo, el gato montés, la marta, la gineta

(*Genetta genetta*), la nutria (*Lutra lutra*), el armiño (*Mustela erminea*), la comadreja (*Mustela nivalis*), el turón (*Mustela putorius*), el zorro (*Vulpes vulpes*) y el tejón (*Meles meles*). El gato montés (Fig. 7A) está presente en todo el parque, aunque su presencia se hace más patente en aquellas cuadrículas UTM que tienen mayor proporción de bosques caducifolios y roquedos, situadas en valles y vaguadas recorridas por arroyos permanentes, y principalmente en la zona de reserva integral (Barja y Bárcena 2005). También la marta europea (Fig. 7B) es más abundante en la zona de reserva integral, donde las perturbaciones humanas son escasas y el hábitat está bien conservado, y ocupa principalmente bosques caducifolios y pinares (Barja 2005b). Entre los ungulados están presentes el corzo (*Capreolus capreolus*), el ciervo (*Cervus elaphus*) y el jabalí (*Sus scrofa*).

El parque cuenta también con una rica y variada comunidad de micromamíferos como demuestran los datos obtenidos mediante los trampeos de vivo (Barja y Piñeiro, datos sin publicar) y el análisis de pelos y restos óseos recolectados en los excrementos de carnívoros como la marta europea y el gato montés (Rosellini et al. 2008, Piñeiro y Barja 2011). Entre las especies de micromamíferos, destaca la presencia del ratón de campo (*Apodemus sylvaticus*) (Fig. 8A), que es la especie más abundante en todos los hábitats, el ratón moruno (*Mus spretus*), el topillo nival (*Chionomys nivalis*), el lirón gris (*Glis glis*), el lirón careto (*Eliomys quercinus*) (Fig. 8B), la musaraña común (*Crocidura russula*), la musaraña de campo (*Crocidura suaveolens*) y el topo ibérico (*Talpa occidentalis*).

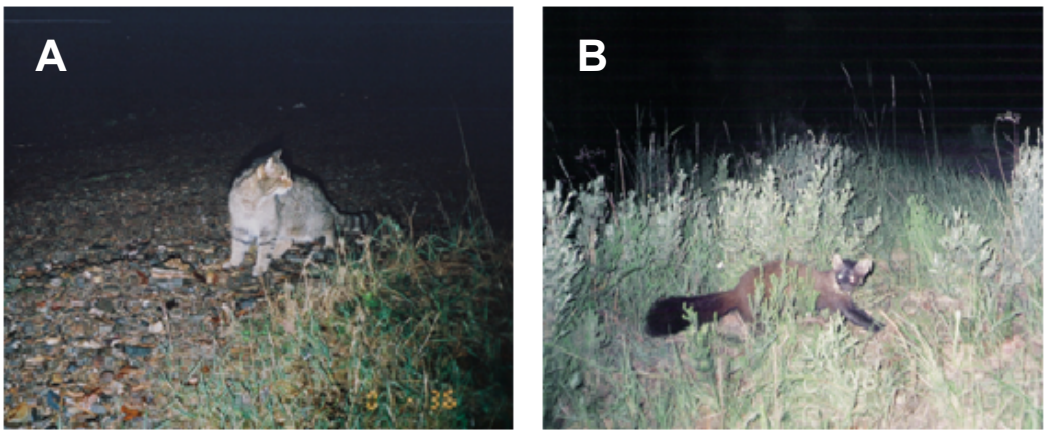


Fig. 7. Gato montés (A) y marta europea (B) fotografiados en el área de estudio mediante el uso de cámaras-trampa. Autora: Isabel Barja.

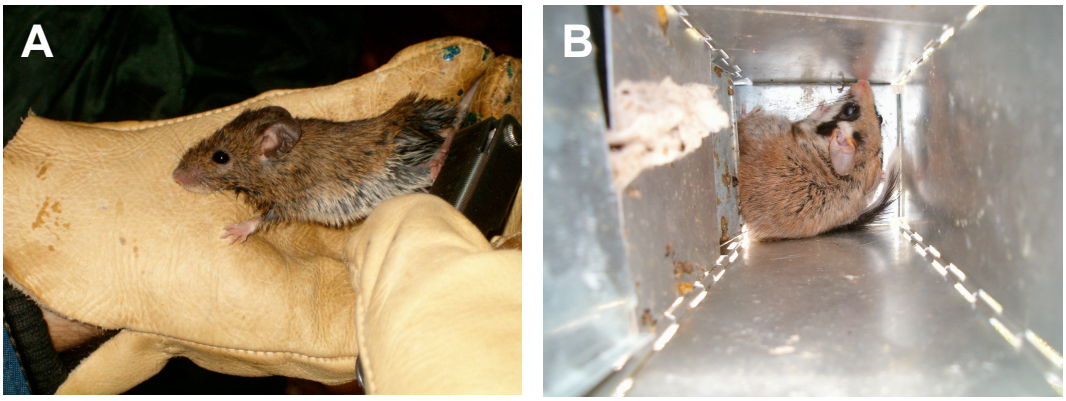


Fig. 8. Ratón de campo (A) y lirón careto (B) fotografiados durante su captura con trampas de vivo Sherman y manipulación para la toma de datos. Autora: Ana Piñeiro.

JUSTIFICACIÓN Y OBJETIVOS

JUSTIFICACIÓN

La mayoría de los estudios científicos realizados con gato montés han analizado aspectos relacionados con su dieta y selección de hábitat. El gato montés es considerado un especialista trófico en el consumo de roedores o conejo, según la región biogeográfica donde se haya realizado el estudio. En el norte de la Península Ibérica la presa principal del gato montés son los roedores, mientras que en el centro y sur es el conejo. A pesar de que en los diferentes estudios el gato montés se define como un especialista trófico en el consumo de roedores o conejo, los autores de los mismos apenas han tenido en cuenta la disponibilidad anual de la presa principal para relacionarla con su consumo. Tampoco existe en la actualidad ningún estudio, excepto el publicado con los datos incluidos en esta tesis doctoral, que considere la vulnerabilidad de captura estacional de la presa principal. Sin embargo, ambos factores son determinantes a la hora de definir la estrategia trófica de cualquier especie y ser tenidos en cuenta en este tipo de estudios.

La mayoría de los carnívoros, incluido el gato montés, son animales nocturnos y solitarios, de ahí que el marcaje oloroso sea su principal forma de comunicación. La comunicación química ofrece ventajas frente a otros canales (visual, táctil, acústica), pudiendo actuar las señales olorosas en ausencia del emisor. El gato montés utiliza orina, secreciones procedentes de glándulas especializadas y heces como marcas oloroso-visuales, las cuales son depositadas sobre diferentes sustratos del medio. El uso preferente de plantas como postes de marcaje en felinos está ampliamente documentado. Sin embargo, en carnívoros apenas existen estudios científicos en los cuales se evalúe qué características físicas de los sustratos les hacen más atractivos como postes de marcaje. El conocimiento de las habilidades de los carnívoros para discriminar entre sustratos de diferente tamaño y diferentes especies vegetales ayuda a avanzar en la comprensión de cómo las especies perciben su entorno. Asimismo, los resultados obtenidos en estudios sobre ecología del comportamiento en carnívoros son de gran utilidad para la gestión del medio en el que viven. En dicha gestión es necesario considerar los patrones de marcaje de cada especie, manteniendo en el medio sustratos que aumenten la probabilidad de detección por otros congéneres al amplificar el componente visual de la señal. La eliminación de sustratos y zonas adecuadas de marcaje oloroso puede influir negativamente en el comportamiento de las especies, cambiando los patrones de uso del espacio.

Las marcas olorosas cumplen funciones importantes en carnívoros, entre las que se pueden mencionar la defensa del territorio, indicación de la condición reproductiva, indicación del estatus social en especies sociales y orientación en el medio, entre otras. La defensa pasiva del territorio mediante señales químicas implica la defensa de recursos (pareja, alimento, refugios, etc) frente a potenciales competidores. Entre los recursos que defienden los carnívoros se encuentran los recursos tróficos, mostrando generalmente los animales un comportamiento de marcaje territorial más acusado en áreas donde el alimento es escaso. Los propietarios del recurso a través de las marcas olorosas intentan minimizar el número de encuentros agresivos con individuos de la misma especie y es probable que también con individuos de otras especies que explotan los mismos recursos tróficos y, por tanto, compiten con ellos. A pesar de la importancia de las señales químicas en el espaciamiento de las especies e individuos, las funciones de las marcas fecales en relación con la defensa del territorio y en particular con la defensa de los recursos que ofrece el mismo permanecen todavía desconocidas en carnívoros.

El gato montés es una especie clasificada como "casi amenazada (NT)" a nivel nacional. Entre las amenazas crecientes que comprometen su futuro se mencionan la destrucción del hábitat, el uso de métodos no selectivos de control de depredadores en los cotos de caza menor, el envenenamiento de sus presas, las excesivas densidades de jabalí y ciervo y, en menor medida, la introgresión de alelos procedentes de gato doméstico. Sin embargo, hay que destacar que en la actualidad las perturbaciones humanas (presión turística, presión cinegética, actividades deportivas, etc.) se encuentran entre las principales causas de la pérdida de biodiversidad, provocando una merma en la calidad de los hábitats que ocupan las especies. Si a esto le añadimos que las actividades de recreo en los espacios naturales protegidos están aumentando vertiginosamente, la condición física individual del gato montés y de otras especies en estas zonas puede verse afectada, lo que sin duda repercute negativamente sobre su capacidad reproductiva, afectando así a su conservación. Además de las actividades humanas, otros factores como la disponibilidad y calidad del alimento, la presencia de otras especies competidoras y el estado reproductor actúan como agentes estresantes en las poblaciones de carnívoros en estado natural. Por estas razones, es necesaria la realización de estudios en los cuales se evalúe qué factores ecológicos y comportamentales son los que determinan cambios en la condición física de las especies en estado natural, utilizando como indicador de la misma los niveles de glucocorticoides (homonas de estrés fisiológico). En los últimos años la

cuantificación de glucocorticoides se ha realizado de forma no invasiva, utilizando muestras fecales, lo que evita la captura y manipulación de los animales para estudiar la respuesta de estrés fisiológico. La recolección de muestras procedentes de heces facilita este tipo de estudios en especies esquivas y con amplios territorios, como ocurre en el caso del gato montés. En vertebrados está bien documentado que cuando un agente estresante actúa durante un periodo corto de tiempo la respuesta de estrés fisiológico ayuda al organismo a superar dicha situación adversa, resultado adaptativa para el organismo. Sin embargo, cuando la situación de estrés es prolongada, los niveles de glucocorticoides se mantienen constantemente elevados, lo que desencadena importantes patologías que afectan a la supervivencia y al éxito reproductivo de las especies y en definitiva a su eficacia biológica. La realización de estudios para evaluar qué factores provocan un aumento en los niveles de glucocorticoides en carnívoros en estado natural favorecerá la conservación de las especies, pues los resultados obtenidos serán de gran utilidad para los gestores de los espacios naturales protegidos al ayudar a tomar decisiones sobre el grado de interacción entre humanos y fauna silvestre, así como para mejorar la gestión de las poblaciones y de sus hábitats.

OBJETIVOS

- **Capítulo 1:** El objetivo de este capítulo es comprobar si el gato montés es una especie especialista trófico en el consumo de ratón de campo (*A. sylvaticus*), su presa principal. Para ello, se analizará si el ratón de campo es la presa seleccionada durante todo el año, a pesar de que en el medio estén disponibles otras presas adecuadas para su consumo. Asimismo, se analizará si el consumo de ratón de campo varía estacionalmente en relación con su disponibilidad en el medio y con su vulnerabilidad para ser capturado.

- **Capítulo 2:** El objetivo es examinar cuáles son las características físicas de los sustratos vegetales que los hacen más adecuados para depositar las marcas fecales por el gato montés. Se comprobará si los gatos depositan las marcas fecales preferentemente en plantas que aumentan la eficacia de la señal, seleccionando aquellos sustratos vegetales de mayor tamaño, los que más destacan en el entorno y determinados grupos de plantas.

Asimismo, se comprobará si los resultados obtenidos apoyan la teoría económica del marcaje propuesta por Roberts y Gosling (2001) para ungulados.

- **Capítulo 3:** Este capítulo fue diseñado para evaluar si las marcas fecales depositadas por los gatos monteses en sus territorios sirven para defender su principal recurso trófico, los roedores. Se evaluará si los gatos depositan un mayor número de marcas fecales en los hábitats donde su presa principal es más abundante (áreas favorables de caza) para proteger su principal recurso trófico y reducir la competencia intraespecífica. Además, se comprobará si las marcas fecales implicadas en la defensa de su principal recurso trófico son depositadas sobre sustratos y zonas que aumenten la probabilidad de detección por otros individuos.

- **Capítulo 4:** En este capítulo se evaluará si la presión turística en un espacio natural protegido y/o el estado reproductor provocan un aumento en los niveles de glucocorticoides fecales en el gato montés. Se examinará si los niveles de metabolitos del cortisol fecal son mayores en las zonas y épocas donde la presión turística es mayor, así como, si estos niveles aumentan durante el celo, la gestación y la dispersión de los individuos jóvenes. Para ello se comprobará si las concentraciones de metabolitos del cortisol fecal están relacionadas con las de las hormonas sexuales (progesterona, estradiol y testosterona).

- **Capítulo 5:** Este capítulo fue diseñado para ampliar el conocimiento sobre cuáles son los factores ecológicos y comportamentales que influyen sobre la respuesta fisiológica de estrés en el gato montés en estado natural. El objetivo es examinar si el tipo de hábitat, la disponibilidad de la presa principal y la competencia interespecífica con la marta europea y el zorro provocan un aumento en los niveles de glucocorticoides fecales en el gato montés. Se comprobará si los gatos monteses muestran niveles de metabolitos del cortisol fecal más elevados en aquellos hábitats donde la presa principal es menos abundante y la competición por interferencia es mayor.

CAPÍTULO 1.

TROPHIC STRATEGY OF THE WILDCAT *FELIS SILVESTRIS* IN RELATION TO SEASONAL VARIATION IN THE AVAILABILITY AND VULNERABILITY TO CAPTURE OF *APODEMUS* MICE

Abstract

The aim of this study was to assess the trophic strategy of the wildcat (*Felis silvestris*) by examining the availability and vulnerability of its main prey. Live traps were used to estimate *Apodemus* mouse availability. The vulnerability to capture of wildcat main prey - *Apodemus* mice - was studied by focal sampling of live-trapped individuals; slow escape behaviour and body weight were used as indicators of vulnerability to capture. The seasonal consumption of *Apodemus* mice did not depend on their availability, although seasonality was the only factor that explained the variation in slow escape behaviour, which was more commonly seen in the autumn when the consumption of these mice was higher. Variation in mouse body weight was related to reproductive condition but not to seasonality. These results indicate that the wildcat is a facultative specialist in the consumption of *Apodemus* mice, with vulnerability to capture the main factor determining the rate of mouse consumption.

Introduction

The composition of the diet of the wildcat (*Felis silvestris*) is one of the best known features of the species' ecology throughout its range (Stahl and Leger 1992, Sunquist and Sunquist 2002, Lozano et al. 2006). Rabbits are the main dietary component in some regions. For example, in eastern Scotland, wildcats feed almost exclusively on rabbits (Corbett 1979), and in the south and centre of Spain rabbits are consumed with greater frequency than rodents (Gil-Sánchez et al. 1999, Malo et al. 2004). Nevertheless, most of the studies conducted in Europe have reported that rodents, especially murines and microtines, are the main dietary items (France: Conde et al. 1972; Caucasus: Nasilov 1972; Italy: Ragni 1978; Carpathians: Kožena 1990; Portugal: Sarmento 1996; Carvalho and Gomes 2004; Spain: Aymerich 1982; Moleón and Gil-Sánchez 2003; Urra 2003). The inclusion of Murinae and Microtinae in the diet also varies with latitude, with higher consumption of the former in southerly locations and higher consumption of the latter in more northerly areas (Lozano et al. 2006).

Most studies suggest that the wildcat is a trophic specialist in the consumption of rodents. A species is considered a trophic specialist when it feeds almost entirely on one prey species or group of prey animals such as "rodents", and when it shows this preference

regardless of the prey's availability or vulnerability to capture (Holling 1959, Glasser 1982). Such predators are said to show a type II (hyperbolic) functional response (Holling 1959). A trophic opportunist, however, consumes the food most at hand in each season and area, changing its diet depending on food availability and vulnerability to capture. When the abundance of one prey type diminishes, opportunist predators begin to take a more abundant species; they therefore show a type III functional response (S-shaped) (Holling 1959, Glasser 1982, Angelstam et al. 1984). A facultative specialist, in contrast, may behave more opportunistically, i.e., by changing a key food item when more profitable prey is available (Glasser 1982).

Prey availability and vulnerability are not key factors in the dietary intake of specialist species; thus, when these factors vary between seasons, no differences should be seen in the wildcat diet. However, trophic ecology studies regarding the quantitative availability of wildcat preys are scarce and no studies have been performed on the vulnerability to capture of its main prey. Although these aspects are essential for defining the trophic strategy of the species, only Malo et al. (2004) have evaluated the trophic strategy of the wildcat in any depth. Nonetheless, in this study the availability and vulnerability of the main prey species (rabbits, and small mammals in areas where rabbits were not available) were not analysed. The present study was designed to test the hypothesis that wildcats are specialist predators of the *Apodemus* mouse. If they are: 1) although other profitable prey species are present in the environment, the *Apodemus* mouse should remain the main prey item year round, 2) *Apodemus* mouse consumption should not vary among seasons according to the environmental availability of the species or its vulnerability to capture.

Material and Methods

Scat collection

Scats were collected seasonally from August 2005 to June 2007 by establishing transects along roads in optimal areas for the species; these areas were identified in previous studies on wildcat distribution and habitat selection in the study area (Barja and Bárcena 2005). In each season we surveyed on foot transects 300 m long in 22 UTM cells of 1 km^2 each (total distance surveyed = 98.4 km in 328 transects). We surveyed in spring 152 trasects, in summer

38, in autumn 72 and in winter 66. These differences between seasons were due to adverse weather conditions at certain months of year. Wildcat mean home range size is of 400 ha (Genovesi and Boitani 1992, Scott et al. 1992) and there is inter-sexual territory overlap (Stahl et al. 1988). Therefore, in order to increase the likelihood of obtaining faecal samples from different individuals and to minimise pseudoreplication, the itineraries were established in four zones of the study area set far apart from one another and in each zone the transects of 300 m were separated by a distance of 1 km. Furthermore, it is important to highlight that faecal sampling on transects avoid the error caused by overrepresentation of particular individuals because increases the probability to obtain samples from different individuals (Huber et al. 2003).

Wildcat scats were differentiated from those of other sympatric carnivores (European pine marten, red fox and badger) by their size and shape. The scats of the wildcat, domestic cat and their hybrids are very similar and their differentiation is complex. However, the human population nearest to the study area is 7 km to the south; the presence of domestic cats would, therefore, at the very least be greatly reduced. During this study, 24 cats were observed and another eight photographed using camera traps; all showed typical wildcat external morphology (Stahl and Leger 1992, Kitchener 1995, Sunquist and Sunquist 2002). Wildcats are reported to show deposit their faeces as marks in conspicuous locations and not bury them (Corbett 1979, Barja and Bárcena 2005), while domestic cats tend to bury their faeces in sympatric zones (Corbett 1979, Schauenberg 1981). None of the faeces detected along the transects were buried; indeed, they often had a marking function, being deposited in prominent locations. This difference in behaviour therefore differentiates their faeces. In addition, recent studies conducted in different European populations on interbreeding between wildcats and feral domestic cats indicate that hybridisation is a problem not very frequent (Randi et al. 2001, Pierpaoli et al. 2003, Lecis et al. 2006, Oliveira et al. 2008b). The presence of domestic cats and hybrids in the study area was therefore not very likely, providing a guarantee of the origin of the scats studied.

Diet analysis

The scats were cleaned in the laboratory following conventional procedures (Reynolds and Aebischer 1991). After drying, the different macroscopic components were separated,

weighed and identified. The consumed prey animals were classified into five categories depending on the indigestible remains: small mammals (rodents and insectivores), other mammals (rabbits and hares), reptiles, birds and insects.

The mammal species were identified from their skulls, jaws and/or teeth using keys (Dueñas and Peris 1985, Blanco 1998). When no bone remains were present, the cuticle patterns of the hairs were compared to those in reference manuals (Faliu et al. 1980, Teerink 1991) and with reference hairs collected in the study area.

The composition of the diet was expressed in terms of frequency of occurrence and percentage of consumed biomass. To estimate the consumed biomass of the five main categories, the following Lockie's correction factors were used (Lockie 1959, Stahl and Leger 1992): small mammals (13.8), reptiles (45), birds (35), insects (5), rabbits (43) and hares (50). The consumed biomass of each prey species was estimated by multiplying its frequency of occurrence by its mean weight (Blanco 1998). At the family and genus levels, consumed biomass was estimated using the mean weight of the corresponding member species.

Availability and vulnerability to capture of Apodemus mice

To estimate the availability of *Apodemus* mice, a live-trapping campaign were performed each season (between August 2005 and June 2007) in the three most representative habitats (deciduous forest, mature pine woods and brushwood). In each habitat, three trapping sites were selected far from one another. A grid containing 25 Sherman traps, separated by 10 m and covering an area of 0.25 ha was defined at each trapping site. In each habitat the traps were left out for three consecutive nights and checked every 12 h. The total effort therefore involved 4,725 trap-nights. Bread impregnated with oil was used as bait.

For each captured *Apodemus* mouse the following data were registered: species, sex, body weight, relative age (individuals weighing less than 15 g were considered juveniles or subadults and those weighing more than 15 g as adults), breeding condition (breeding or non-breeding individuals) and habitat type (deciduous forest, mature pine wood and brushwood). The mice were reliably sexed and their breeding status determined on the basis of several indicators (Gurnel and Flowerdew 1994). All the captured individuals were marked with inoffensive paint to differentiate them in later recapturing.

Slow escape behaviour (reflecting the vulnerability to capture by wildcats) of 104 *Apodemus* mice (71 adults, 18 subadults, 7 young, 8 of undetermined age) was recorded by focal sampling (one-zero recording) (Martin and Bateson 1986). Every focal animal was observed at 5 s intervals over 2 min. These live-trapped individuals were not freed directly from the traps, first were handled (weighed, sexed, marked and observed their breeding condition), to avoid that hypothermia during cold seasons affected the results. The handling time of each animal was approximately 5 min. After the handling each individual was released at the place of capture, but in an area free of vegetation (to aid visibility), and the slow escape behaviour recorded. Although the number of recaptures was low (12.5%) during the experiment, we did not consider them for statistical analysis in order to avoid bias related with the fact that recaptured animals are more stressed than those captured for the first time and also to avoid problems of pseudoreplication.

Data analysis

To determine the relationship between the consumption of *Apodemus* mice by wildcats and their seasonal abundance, the number of scats containing *Apodemus* remains every season (ObsF) was recorded. Since the scat number collected in each season was different, the figures were corrected using the equation:

$$\mathbf{ObsF^* = ObsF \times I_c;} \quad \mathbf{I_c} = \overline{N}/\mathbf{N} \qquad (1)$$

where ObsF* is the corrected number of scats with *Apodemus* remains, I_c is the correction index, N is the total number of scats analysed per season, and $\overline{N}$ the annual mean number of scats analysed.

The expected percentages of scats with *Apodemus* remains (ExpF%) in each season were calculated according to the following formula:

$$\mathbf{ExpF\% = A_s \times 100/ A_t} \qquad (2)$$

where A_s is the seasonal number of captured *Apodemus* and A_t the total number of captured *Apodemus* in the study area.

The expected frequencies (ExpF) of scats with *Apodemus* remains per season were calculated with the following formula:

$$\mathbf{ExpF = \sum ObsF^* \times ExpF\%/100} \qquad (3)$$

To compare the overall dietary diversity (trophic niche breadth) in different seasons, the *B* index of Levins (1968), which varies from 1 (narrowest niche) to 5 (broadest possible niche), was calculated for the five food categories. The index was applied to percentage of occurrence of the main food categories (small mammals, reptiles, insects, birds, other mammals) in the whole diet.

Slow escape behaviour and the body weight of the individuals (dependent variables) were analysed via general linear models. In both models the following variables were used as fixed factors: season (spring: April-June, summer: July-September, autumn: October-December, winter: January-March), sex, age (adults, young or subadults), reproductive condition (breeding or non-breeding) and habitat (deciduous forest, mature pinewood or brushwood). Turkey's *post hoc* test was used for the pairwise comparison of seasonal means. Significance was set at $P < 0.05$. All calculations were performed using SPSS v. 15.0 software for Windows (SPSS Inc, Chicago, IL, U.S.A.).

Results

General remarks

A total of 200 wildcat scats (422 prey items) were used in the dietary analysis (spring: 27, summer: 60, autumn: 55 and winter: 58). The mean number of prey animals per scat was of 2.3. Small mammals were the most important prey item (frequency of occurrence 85.6%), followed by reptiles (5.9%), insects (4.7%), other mammals (1.9%) and birds (1.9%) (Table 1) ($\chi^2 = 1119.9$, df = 4, $P = 0.0001$, $N = 422$).

Among the mammals, rodents (frequency of occurrence 90.0%) were consumed in preference to insectivores (7.8%) and other mammals (rabbits and hares) (2.2%) ($\chi^2 = 534.5$, df = 2, $P = 0.0001$, $N = 369$). The consumption of Murinae was significantly higher than that of Microtinae (frequency of occurrence 80.7% compared to 19.3%) ($\chi^2 = 99.4$, df = 1, $P = 0.0001$, $N = 264$), with *Apodemus* being the genus most preyed upon (reaching a frequency of occurrence for all Murinae eaten of 61.0%) (Table 1) ($\chi^2 = 9.0$, df = 1, $P = 0.003$, $N = 187$).

Table 1. Diet composition of wildcats in the northwestern Iberian Peninsula. [1]FO: frequency of prey occurrence in scats. [2] CB%: percentage of consumed biomass.

		Genre/Specie	FO[1]	CB%[2]
Mammals	Rodents	Murinae	26	3.2
		Apodemus sp. (*A. sylvaticus* and *A. flavicollis*)	114	24.0
		Mus sp.	73	5.1
		Arvicola sp.	5	3.8
		Water vole (*Arvicola terrestris*)	2	0.8
		Microtus sp.	27	4.2
		Field vole (*Microtus agrestis*)	1	0.2
		Cabrera vole (*Microtus cabrerae*)	2	0.6
		Lusitanian pine vole (*Microtus lusitanicus*)	4	0.4
		Bank vole (*Clethrionomys glareolus*)	4	0.5
		Snow voles (*Chionomys nivalis*)	6	2.3
		Fat dormouse (*Glis glis*)	29	2.1
		Garden dormouse (*Eliomys quercinus*)	13	3.2
		Red squirrel (*Sciurus vulgaris*)	26	38.0
		Total items	**332**	**88.4**
	Insectivores	*Crocidura* sp.	8	0.3
		Greater white-toothed shrew (*Crocidura russula*)	1	0.0
		Sorex sp.	10	0.5
		Pygmy white-toothed shrew (*Suncus etruscus*)	2	0.0
		Water shrew (*Neomys fodiens*)	7	0.5
		Iberian mole (*Talpa occidentalis*)	1	0.3
		Total items	**29**	**1.6**
	Other mammals	Rabbit (*Oryctolagus cuniculus)*	7	1.6
		Hare (*Lepus granatensis*)	1	0.3
Birds			8	1.5
Reptiles			25	6.1
Insects			20	0.5
Nº scats analysed			200	

Seasonal patterns

The consumption of small mammals showed seasonal variation, with more being taken in the autumn (frequency of occurrence 95.0%) and winter (90.1%) than in the spring (78.5%) or summer (76.4%) (Table 2) (χ^2 = 174.0; df = 3, P = 0.0001, N = 361). The highest consumption of reptiles occurred during summer (11.0%) and spring (9.2%), followed by winter (2.7%) and autumn (1.7%) (χ^2 = 14.2, df = 3, P = 0.003, N = 25). The consumption of birds and other mammals also experienced seasonal variation, becoming significantly higher in winter (3.7%) and spring (6.2%). Although insects made up occasional prey items, these were better represented in the summer diet (frequency of occurrence 9.4%) than in other seasons (Table 2). Taking into account the main prey categories, the trophic niche breadth (B) was higher in the hot seasons (spring: 2.5, summer: 2.2) than in the cold seasons (autumn: 1.2, winter: 1.5).

Table 2. Seasonal variation in wildcat prey consumption (five categories) and trophic niche breadth (Levins index). N: Number of scats, FO: frequency of prey occurrence in scats, CB%: percentage of consumed biomass.

			Small mammals		Reptiles		Insects		Birds		Other mammals	
Seasons	**N**	**Levins index**	**FO**	**CB%**	**FO**	**CB%**	**FO**	**CB%**	**FO**	**CB%**	**FO**	**CB%**
Spring	27	2.5	51	56.2	6	25.6	3	1.2	1	2.9	4	14.1
Summer	60	2.2	97	60.0	14	30.6	12	2.8	3	4.7	1	1.9
Autumn	55	1.2	113	89.7	2	5.2	2	0.6	1	2.0	1	2.5
Winter	58	1.5	100	79.8	3	7.8	3	0.9	3	6.1	2	5.4

In spring, *Apodemus* was the most consumed prey item, followed by *Mus*, *Microtus*, *Arvicola*, *Sciurus*, *Clethrionomys* and *Glis* (χ^2 = 35.5, df = 6, P = 0.0001, N = 37). In autumn, *Apodemus* and *Mus* were the main prey, followed by *Glis*, *Microtus*, *Eliomys*, *Sciurus*, *Arvicola*, *Chionomys* and *Clethrionomys* (χ^2 = 94.9, df = 8, P = 0.000, N = 104). In summer, *Apodemus* was more commonly consumed than *Mus* and, to a lesser extent, the wildcats preyed

on *Sciurus*, *Eliomys*, *Glis*, *Microtus*, *Arvicola*, *Chionomys* and *Clethrionomys* ($\chi^2 = 87.1$, df = 8, $P = 0.000$, $N = 81$). In winter *Apodemus* was also the most common prey item, followed by *Mus*, *Microtus*, *Sciurus* (11.9%), *Glis*, *Chionomys* and *Eliomys* (Table 3) ($\chi^2 = 63.5$, df = 6, $P = 0.000$, $N = 84$).

Table 3. Seasonal variation in the consumption of different genus of small mammals (%) by wildcats. Apo: *Apodemus*; Mus: *Mus*; Micr: *Microtus*; Arv: *Arvicola*; Sci: *Sciurus*; Clet: *Clethrionomys*; Chio: *Chionomys*; Eli: *Eliomys* and Gli: *Glis*.

Seasons	N	Apo	Mus	Micr	Arv	Sci	Clet	Chio	Eli	Gli	Total
Spring	37	37.9	32.4	16.2	5.4	2.7	2.7	0	0	2.7	100
Summer	81	38.3	22.2	5	2.5	13.6	1.2	1.2	8.6	1.2	100
Autumn	104	35.6	20.2	12.5	2.9	3.8	1.9	2.9	4.8	15.4	100
Winter	84	38.1	26.2	13.1	0	11.9	0	2.4	1.2	7.1	100

Availability of Apodemus mice

A total of 232 mice belonging to the genus *Apodemus* were captured during the live trapping campaign. The abundance of mice was higher in summer (30.6%) and autumn (26.7%) than in winter (21.6%) or spring (21.1%) (Table 4), although these differences were not significant ($\chi^2 = 5.7$, df = 3, $P = 0.128$, $N = 232$). More adult individuals (>15 g) were captured in summer (31.2%) and spring (24.7%) than in autumn (23.7%) or winter (20.4%) (Fig. 9), but these differences were not significant either ($\chi^2 = 2.3$, df = 3, $P = 0.519$, $N = 93$). However, young individuals (< 15 g) were more abundant in autumn (38.0%) and winter (24.0%) than in spring (20.0%) or summer (18.0%) (Fig. 9) ($\chi^2 = 4.9$, df = 3, $P = 0.181$, $N = 50$).

Table 4. Seasonal variation in the consumption of *Apodemus* mice (observed frequencies) by wildcats and their expected consumption (expected frequencies) in relation to their environmental availability (see Method section). N: total number of scats analysed per season. ObsF: number of scats with *Apodemus* remains. I_c: applied correction index. ObsF*: corrected number of scats with *Apodemus* remains. ObsF%*: corrected observed percentages of scats with *Apodemus* remains. ExpF: expected frequencies of scats with *Apodemus* remains. ExpF%: expected percentages of scats with *Apodemus* remains. A: abundance of *Apodemus* in the environment.

	N	ObsF	I_c	ObsF*	ObsF%*	ExpF	ExpF%	A
Spring	27	14	1.85	25.9	23.2	23.6	21.1	49
Summer	60	31	0.83	24.7	22.1	34.2	30.6	71
Autumn	55	37	0.91	33.7	30.1	29.9	26.7	62
Winter	58	32	0.86	27.5	24.6	24.1	21.6	50
Total	200	114		111.8	100	111.8	100	232

The number of breeding individuals of *Apodemus* was significantly higher in the hot seasons (spring and summer) (86.5%) than in the cold seasons (autumn and winter) (13.5%) (χ^2 = 19.7, df = 1, P = 0.000, N = 37). The number of non-breeding individuals of *Apodemus* was higher in the cold seasons (66.3%) than in the hot seasons (33.7%) (χ^2 = 10.8, df = 1, P = 0.001, N = 101).

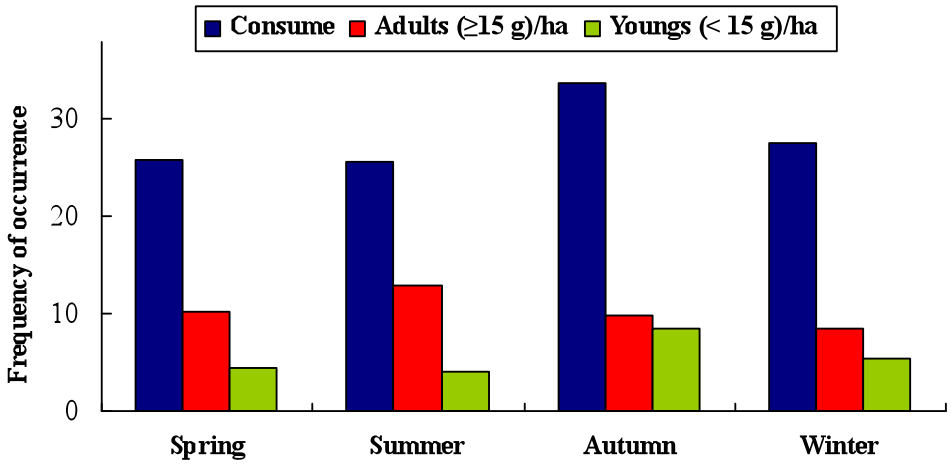


Fig. 9. Number of wildcat scats with *Apodemus* remains by seasons and compared to the abundance of adult and young *Apodemus* mice in the environment.

Consumption of *Apodemus* mice in relation to their availability and vulnerability to capture

The seasonal consumption of *Apodemus* mice did not depend on their availability. In the spring (23.2 vs. 21.1%), autumn (30.1 vs. 26.7%) and winter (24.6 vs. 21.6%), *Apodemus* mice were more frequently consumed than expected for their availability (Table 1). However, in summer they were consumed at a frequency lower than expected for their availability (22.1 vs. 30.6%) (Table 1, Fig. 9).

The general linear model indicated seasonality to be the only factor explaining the variation in slow escape behaviour. Slow escape behaviour was more frequently showed in autumn (13.2 ± 4.2 s), followed by winter (7.7 ± 2.3 s), spring (7.5 ± 2.1 s) and summer (4.4 ± 1.5 s) (Fig. 10) (factorial ANOVA: F = 3.876, df = 3, $P = 0.018$, $N = 104$). The only factor that explained the weight variation of the mice was their breeding condition (breeding: 25.8 ± 0.8 g, non-breeding: 23.9 ± 0.7 g) (factorial ANOVA: F = 4.577, df = 1, $P = 0.038$, $N = 104$), but weight did not vary markedly with season (spring: 24.2 ± 1.4 g, summer: 23.1 ± 0.9 g, autumn: 21.7 ± 1.3 g, and winter: 20.6 ± 1.1 g) (factorial ANOVA: F = 0.540, df = 3, $P = 0.657$, $N = 104$).

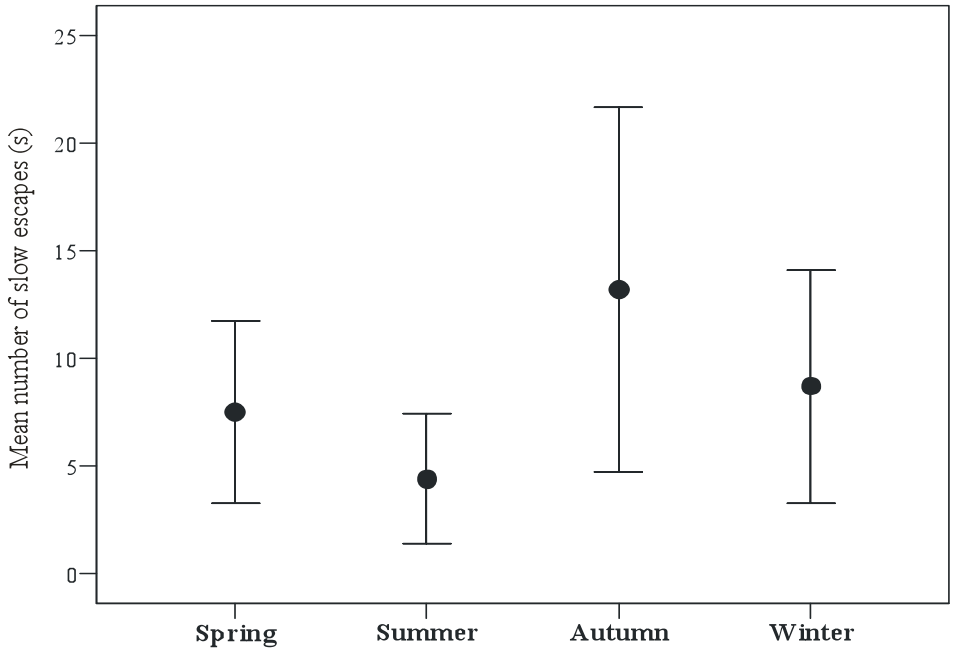


Fig. 10. Slow escape behaviour in *Apodemus* mice per season (mean ± SE).

Discussion

This study showed that rodents, mainly *Apodemus* mice, are the wildcat's most common prey species in the study area. These results agree with those reported in previous studies from other regions of the Iberian Peninsula (Aymerich 1982, Sarmento 1996, Moleón and Gil-Sánchez 2003, Carvalho and Gomes 2004) and Europe (Condé et al. 1972, Ragni 1978, Kožená 1990, Liberek 1999, Biró et al. 2005). In Mediterranean areas, where the density of rabbits is low or nil, wildcats select rodents, as occurs in many Eurosiberian regions of Europe (Kožená 1990, Sarmento 1996, Moleón and Gil-Sánchez 2003, Malo et al. 2004, Biró et al. 2005). Nevertheless, when rabbits are abundant, they become the main prey species (Malo et al. 2004).

In the present study, the seasonal consumption of *Apodemus* mice did not depend on the environmental availability of this prey item. In autumn, winter and spring wildcats consumed more *Apodemus* mice than would be expected for their availability. In summer, however, this prey species was negatively selected, its consumption being lower than that expected for its availability. These results disagree with those reported by Stahl (1986) and Stahl and Leger (1992), who indicated that the consumption of rodents by the wildcat depends on the formers' temporal and spatial availability in the environment.

The present results show that there were more young *Apodemus* mice in the environment in autumn and winter, therefore, we hypothesize that their scant anti-predator experience makes their capture easy for predators. The individuals that survived in these seasons would be weaker, less agile and therefore less likely to reach their burrows before being caught by a predator.

In summer, the lower-than-expected consumption of *Apodemus* mice might be explained by the higher trophic diversity on offer to wildcats; prey items absent or scarce in the other seasons (e.g., insects), or that might be difficult to detect and capture at other times of year (e.g., hibernating reptiles) would be more abundant. The taking of these two prey items (reptiles and insects) may provide an advantage in terms of energy optimisation.

Catchability is a key parameter in prey selection (Stephens and Krebs 1986). One anti-predator strategy of prey animals consists of simply running away from their predators (Lima and Dill 1990), but this capacity can be diminished at certain times of year due to a shortage of food resources or adverse meteorological conditions, both of which can weaken prey animals.

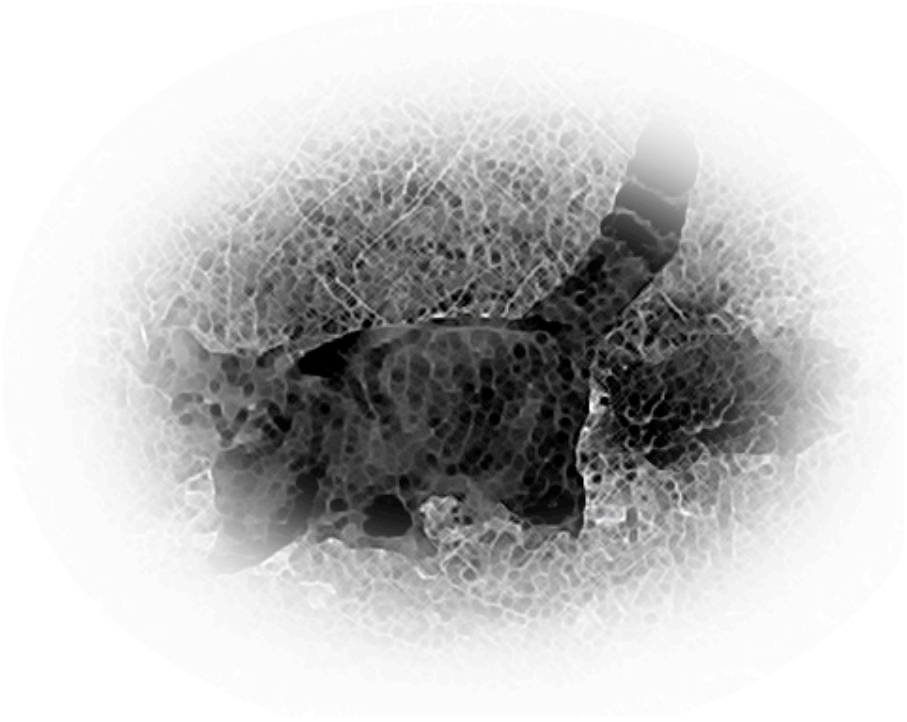
In the present study, the *Apodemus* mice (a basic resource for wildcats and other medium sized carnivores such as the pine marten [e.g. see Rosellini et al. 2008]), showed slower escape behaviour during the autumn and winter. Thus, at this time of the year the probability of their capture by predators is at its highest; prey capture success is therefore greater and at lower cost to the predator (Caraco 1980, Williams and Nichols 1984, Belovsky et al. 1989). Wildcats seem to synchronise the consumption of their main prey with the season when it is most vulnerable. The results seem to show that the population of *Apodemus* in autumn to be made up of generally younger or thinner individuals and of others not in their top physical condition, i.e., animals less likely to be able to avoid predators.

During the cold seasons the mice could be weaker due to the high energy costs of the past reproductive period (spring and summer in the study area) and the effort used in the searching for food and refuge. In another hand, the wounds inflicted by dominant males on their subordinates during the reproductive season might also limit the survival chances of the latter (Torre et al. 2002). In autumn and winter, when the *Apodemus* slow escape time was longer, the wildcat handling time should decrease because the pursuit phase is shorter, meaning a better energy balance for the predator (Kacelnik and Bernstein 1994).

The results therefore suggest that the wildcats of the study area follow a facultative trophic strategy with respect to the consumption of *Apodemus* mice, their vulnerability to capture being the main factor that determines the pattern of their consumption. The same conclusion has recently been reached by Malo et al. (2004) in a study conducted in the centre of the Iberian Peninsula. Nevertheless, these authors did not control for the abundance of rodents and rabbits (only for their presence or absence), nor their vulnerability to capture.

The idea of facultative specialization proposed in this study is supported by the following results: (1) dietary diversity was greater in the hot seasons (spring and summer) than in the cold seasons (autumn and winter); in the hot seasons, the wildcats shifted their diet from *Apodemus* mice to other available prey species such as reptiles, insects and birds; (2) significant variations in small mammal consumption was seen among seasons, supporting the idea that the wildcats in the study area are not specialist species; and (3) at certain times of year the wildcats preyed more than expected on *Apodemus* mice in relation to their environmental availability, probably because their detection, capture and manipulation were easier. These results would not be expected of a trophic specialist species.

CAPÍTULO 2.
PLANT PHYSICAL FEATURES SELECTED BY WILDCATS AS SIGNAL POSTS: AN ECONOMIC APPROACH TO FECAL-MARKING



Abstract

Chemical signals of solitary and territorial felid species are essential for their intraspecific communication. We studied the selection of vegetal substrates during the fecal marking behaviour of the European wildcat *Felis silvestris* from September 2008 to June 2009 in a protected area in Northwest Spain. The aim of the study was to examine the selection of plants as signal posts with respect to their physical characteristics. We hypothesized that wildcats deposit their fecal marks on plants with physical characteristics (e.g., size, species, and visually conspicuousness) that enhanced the olfactory and visual efficiency of the signal. Our results indicate that diameter, vegetal group, visual conspicuousness, and interaction between the diameter and vegetal group influence the decision of wildcats to deposit their fecal marks on plants. Wildcats chose plants with greater diameters and greater visual conspicuousness as scent-marking posts. Moreover, the wildcats chose woody and herbaceous plants, and certain plant species were marked more frequently than expected at random. Our results indicate that fecal marks were not randomly distributed on plants; they were placed on plants whose physical characteristics maximized the detectability of the signal by intruders and potential mates, facilitating the spacing of the species.

Introduction

Carnivores use different types of signals to communicate, including acoustic, tactile, visual, and chemical signals (Eisenberg and Kleiman 1972). However, scent marks are advantageous because they can be used where other signals may be difficult to detect, such as in dense vegetation, at night, or underground (Gorman and Trowbridge 1989). In addition, such signals can remain active for a long time so that other animals can smell individual signals even when the signaler is absent (Gosling and Roberts 2001).

Carnivores, particularly felids, use urine, glandular secretions, and feces as chemical signals (Brown and Macdonald 1985, Robinson and Delibes 1988). The roles that these scent marks may play are well known: territory defense (Eaton 1970a, Smith et al. 1989),

indicators of reproductive status (Wemmer and Scow 1977, Molteno et al. 1998, Richardson 1998), individual identification (Wemmer and Scow 1977), preventing agonistic encounters (Smith et al. 1989), indicators of social status (Ralls 1971), and immune and physiological condition (Zala et al. 2004). Nevertheless, despite the relevance of chemical signals in the lives of carnivores, few studies on chemical communication in felids have been conducted; some authors merely refer to this behaviour in studies focusing on other aspects of their ecology. Most of the existing studies reference urine marking (Smith et al. 1989, Bothma and le Riche 1995, Molteno et al. 1998, Andersen and Vulpius 1999); only 2 articles have been published on fecal marking (Iberian lynx, *Felis pardina*: Robinson and Delibes 1988; Geoffroy's cat, *Leopardus geoffroyi*: Soler et al. 2009).

Although most felids are solitary animals, their lives are embedded in a social system that is dependent on the presence of signaling systems for regulation of the animals' interactions (Sunquist and Sunquist 2002); therefore, chemical signals are essential in their intraspecific communication. The European wildcat (*Felis silvestris*) is a solitary and crepuscular animal whose encounters with other congeners are mainly limited during the breeding season (Kitchener 1995, Sunquist and Sunquist 2002). In addition, this felid is a territorial carnivore, in which males typically have home ranges that overlap with those of multiple females (Corbett 1979, Stahl et al. 1988). Therefore, wildcats use scent marks to proclaim their territorial boundaries (Stahl and Leger 1992) as a strategy when the costs of patrolling the border are high, as is the case in many other mammals (Gosling 1982).

The production and maintenance of scent marks incurs costs (Gosling and Roberts 2001). For this reason, carnivores frequently deposit scent marks in places that increase their effectiveness as visual and olfactory signals (Peters and Mech 1975, Vilà et al. 1994, Barja et al. 2004, 2005, Barja 2009). Similarly, Gosling (1981) proposes an economic approach of scent marking in ungulates, which predicts that scent marks should be placed in zones that maximize the chances of being detected by conspecifics. Therefore, chemical signals should not be distributed randomly but should be placed at strategic sites (e.g., visually conspicuous substrates, above ground level, or at crossroads) to maximize their detectability and increase the active space of chemical components (Alberts 1992, Gosling and Roberts 2001). Many animals use local landmarks as points of reference to create internal maps of the environment

during their spatial navigation (Etienne et al. 1999). Local landmarks are often prominent or visually distinct objects (e.g. shrubs or logs) that allow precise encoding of a location (Cheng and Spetch 1998). However, when environmental cues are unavailable or in absence of light, scent marks can be used as trail marks (Lavenex and Schenk 1996, De León et al. 2003, Kulvicius et al. 2008).

Plants are often used as scent-marking posts by different carnivore species (cheetah, *Acinonyx jubatus*: Eaton 1970a; wildcat: Corbett 1979; tiger, *Panthera tigris*: Smith et al. 1989; leopard, *Panthera pardus*: Bothma and le Riche 1995; Iberian wolf, *Canis lupus*: Barja 2009; African civet, *Civettictis civetta*: Tsegaye et al. 2008, Wondmagegne et al. 2011). The use of plants as substrates to deposit scent marks seems to confer certain advantages over deposition on the ground or other types of substrates. In this sense, Smith et al. (1989) showed that urine marks are detectable for a longer period when deposited on vegetal substrates than on the ground. However, despite the advantages of vegetal substrates as marking posts, few studies have been performed on mammalian species to determine the physical characteristics of plants that are more suitable for depositing scent marks (tiger: Smith et al. 1989; klipspringer, *Oreotragus oreotragus*: Roberts 1997; African civet: Tsegaye et al. 2008; Iberian wolf: Barja 2009). Furthermore, to date, few published studies are available on the ability of carnivores to discriminate plants by species and size during scent marking behaviour; such information would help us to know more about how these animals perceive their environment (Barja 2009).

Wildcats frequently deposit scent marks on plants (Corbett 1979), which could be because of the abundance of these plants in the wildcats' habitat or the benefits of these vegetal substrates to deposit scent marks, as suggested in other mammals (Roberts 1997, Barja 2009). Thus, not all plants seem to be equally suitable for depositing scent marks. Therefore, wildcats should deposit feces on plants that increase the efficiency of the signal. If this true, it is possible to predict that wildcats will select larger, more apparent plants.

Materials and methods

Wildcat feces survey

The field data collection was performed from September 2008 to June 2009. Wildcats (Corbett 1979) and other felids (Iberian lynx: Robinson and Delibes 1988; ocelot, *Felis pardalis*: Emmons 1988; cheetahs: Broomhall et al. 2003) often use roads for travelling and frequently defecate on them as a means of scent marking. Therefore, we established a total of 9 routes along roads, ranging in length from 1 to 12 km. The routes were separated from each other by an average distance of 3.4 km (range, 0.5-6.8 km). The routes were established in the most optimal zones for the wildcat based on the previous studies on distribution and habitat selection in the same study area (Barja and Bárcena 2005). In each route, 300-m-long transects were surveyed. The transects were separated from each other by 700 m, and a total of 31 transects were surveyed monthly. All transects included in the routes were conducted on foot to locate fresh wildcat scats deposited on plants.

Specific, individual, and sexual identification of fecal samples

Because of their shape and size, wildcat feces are difficult to distinguish from those of other medium-size carnivores, mainly feral cats and hybrids. Therefore, it is necessary to use a multifaceted approach involving DNA methods, as reported Davison et al. (2002), to distinguish between the feces of different felid species.

Specific identification through molecular analysis was conducted to determine the specific origin (i.e., wildcats rather than feral cats, hybrids or other carnivores) of the scats and the reliability of the obtained data. Individual genotyping was performed as described by Oliveira et al. (2009) to determine the minimum number of individual wildcats from which the scats originated; this information was necessary to determine whether the number of detected scats was representative of the wildcat population and to minimize pseudoreplication. To perform specific identification and individual genotyping, we collected 26 fresh fecal samples spread evenly throughout the study area. In addition, these fecal samples were sexed and individually identified by visualizing 2 samples under low

amplification to identify the sex. A total of 16 different wildcat genotypes were identified including 5 males and 11 females.

DNA was extracted from fecal samples by salting-out and phenol-chloroform extraction (Sambrook et al. 1989). The individual multilocus genotypes were assessed using 12 neutral unlinked microsatellites that were previously isolated and characterized in the domestic cat (Oliveira et al. 2008a). Polymerase chain reaction (PCR) amplification of individual microsatellites was performed according to the procedure given by Randi et al. (2001). Allele frequencies, standard diversity indices, and observed (H_O) and expected (H_E) heterozygosities for each locus and population were calculated using GENETIX 4.05 (Belkhir et al. 1996-2004).

Reliotype (Miller et al. 2002) was used to assess the reliability of our multilocus genotypes and to estimate the number of replicates necessary for obtaining a genotype with 95% confidence. GIMLET (Valiere 2002) was used to estimate error rates and construct consensus genotypes from the repeated genotyping; moreover, it was used to regroup identical genotypes from different scat samples and determine parentage between individuals (kinship).

Plant physical characteristics

To determine the characteristics of plants that are crucial in the decision-making process of wildcats to deposit fecal marks, every time a fresh scat was located on a vegetal substrate, the plant species was identified, and its maximum height and diameter were measured (Table 5).

Table 5. Variables related with the plant physical characteristics considered as potential factors influencing fecal-marking behaviour on plants in wildcats.

Variables	Definition	Hypothesis
VEGETAL GROUP (herbaceous/pulse/woody)	We clasified the plants in three categories: 1) Herbaceous: false brome, *Brachypodium sylvaticum*. 2) Pulse: tall oatgrass, *Arrhenatherum elatius*, yorkshire fog, *Holcus lanatus* and quacking grass, *Briza media*. 3) Woody: broom, prickled broom, bell heather, *Erica cinerea*, blackberry, *Rubus sp*. and yellow rock rose.	Some carnivores selected plants to deposit scent marks in relation to their specific characteristics, which seem increase the detection probability of the mark and the persistence in time (Schaller et al. 1985, Clevenger and Purroy 1991, Barja 2009). Therefore, the wildcats will choose those plant species that best enhance the effectiveness of fecal marks.
VISUAL CONSPICUOSNESS (conspicuous/ inconspicuous)	We have considered that a scat was on a visually conspicuous plant when that one was the most notorious to a human observer within a circle with 2 m radius, with the plant at the centre (Barja 2009).	The scent marks are expected to be placed in visual conspicuous substrates in order to maximise their detectability by other animals (Gosling and Roberts 2001), as observed in other carnivores (Macdonald 1980, Barja et al. 2005, Barja 2009).
HEIGHT AND DIAMETER (cm)	We have measured the maximum diameter and height of the plants.	Plants with greater height and diameter is expected to be more selected to deposit the scent marks. These substrates enhance the scent component of the signal, thus facilitating high marks to be the release of volatile compounds (Alberts 1992) and also enhance the visual component, thereby increasing their effectiveness.

In addition, to estimate the availability of different sizes and species of plants in the environment and to determine whether there was indeed a selection process, 196 plots (1 × 3 m) were established on the roads where transects were performed. The plots were distributed evenly throughout the study area. In each plot, plant species and maximum height and diameter were recorded for the 4 largest plants (Table 5). In addition, the visual conspicuousness of plants both marked and unmarked by feces was determined (Table 5).

Data analysis

A generalized linear mixed model (GLMM) was used to examine the relationships between the presence/absence of fecal marks on plants and different predictor variables as follows: (1) factors, vegetal group and visual conspicuousness and (2) covariates, height and diameter. The dependent variable "fecal marking/non-fecal marking on plants" was modeled with a binomial error distribution and a logit link function. The Akaike Information Criterion (AIC) (Burnham and Anderson 2002) was used to select the most parsimonious models. To select the best GLMM models, the Akaike weights of each model were estimated by following the procedures given by Burnham and Anderson (2002). The variables with the highest weight ($\Sigma\omega_m = 0.95$) were considered more important than others. Candidate models were selected according to the rule in which models with $\Delta i \leq 2$ were considered to have substantial empirical support (Burnham and Anderson 2002).

Different plant species were pooled into 3 categories for statistical analysis: herbaceous, pulse, and woody plants (Table 5). Jacobs' selection index (D) was estimated for each vegetal group for determining the groups of plants selected by wildcats to deposit fecal marks and those that were avoided. Jacobs' selection index ranges from -1 (total avoidance) to 1 (strongest preference); a value of 0 indicates random selection (i.e., the vegetal group was selected according to its availability) (Jacobs 1974).

The chi-squared test (χ^2) was used to analyze the differences between observed frequencies (use of plants) and expected frequencies (availability of plants) with respect to their conspicuousness, vegetal group, and species.

The height and diameter of the plants marked with feces and availability of plants in the study area are expressed as means ± standard error (SE). The level of significance was set

at $P < 0.05$. All analyses were performed with STATISTICA v.8.0 software for Windows (StatSoft Inc., Tulsa, OK, U.S.A.).

Results

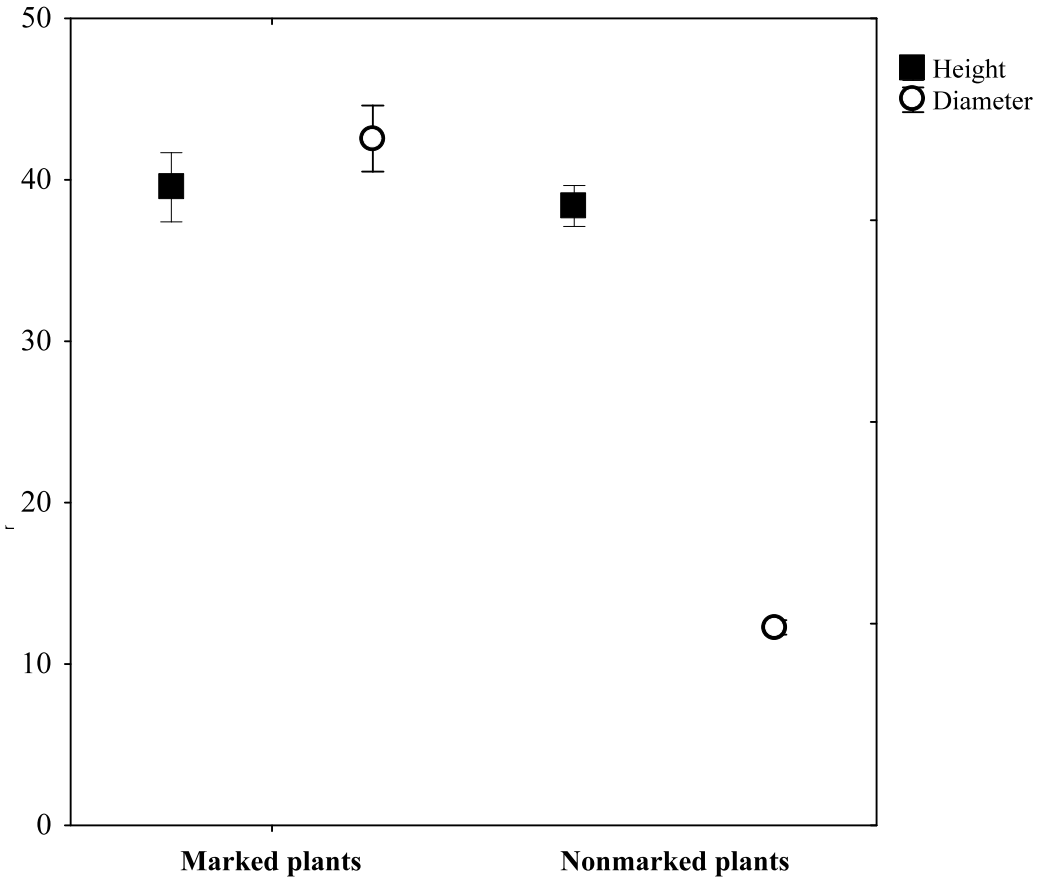
During the study, a total of 104 fresh wildcat scats were found on plants. The physical characteristics of 519 plants available in the environment (not used for depositing fecal marks) were also analyzed.

The model selection process for fecal marking on plants indicated that 31 occurrence models and 6 models were regarded as plausible. Diameter and vegetal group were given the most importance in the process of selecting occurrence models, with both having positive values (Table 6). Thus, plants marked more frequently with feces by wildcats had greater diameters and belonged to the groups of herbaceous and woody plants. The mean diameter of feces-marked plants was greater than that of unmarked plants, while the mean height of feces-marked plants was similar to that of unmarked plants (Fig. 11). The diameter × vegetal group interaction was another robust finding (Table 6).

Table 6. Results of generalized linear mixed model (GLMM) averaging inference for occurrence of fecal marks on plants in wildcats. The table shows for each variable the weight across of the most parsimonious models ($\sum w_i$), β coefficient and standard error (SE).

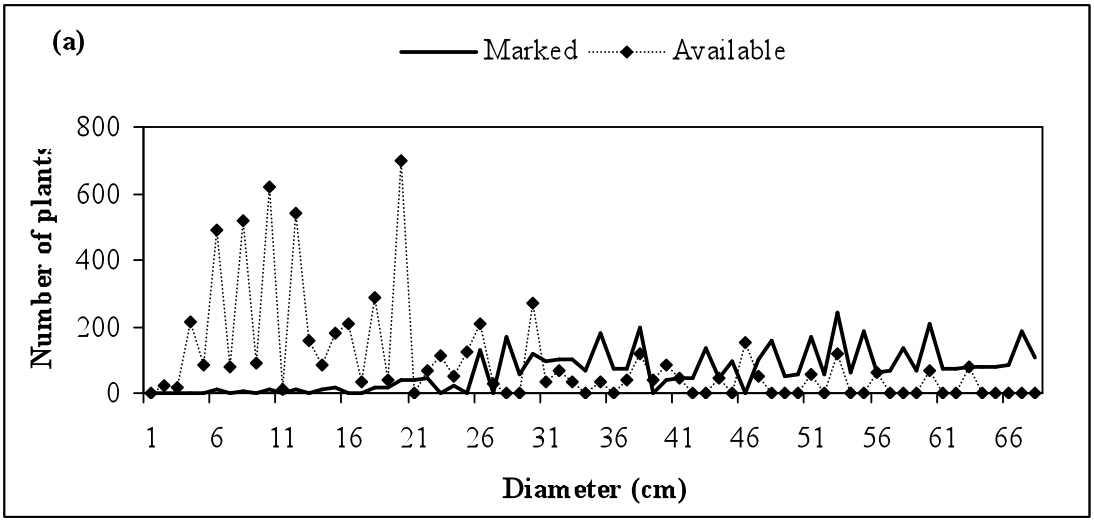
Variable	$\sum w_i$	β	SE
Intercept		99.72	29.61
Diameter	1.00	69.87	8.93
Vegetal group	1.00	8.04	8.93
Visual conspicuousness	0.57	0.52	0.85
Diameter * Vegetal group	0.30	8.99	10.78

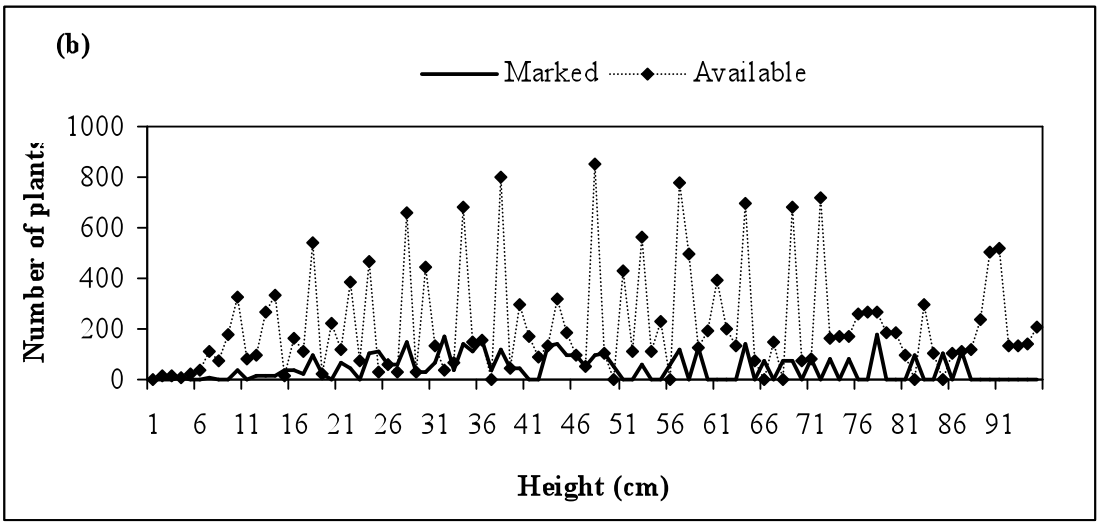
Fig. 11. Comparison of mean height and diameter of feces-marked plants (N = 104) and non-marked plants (N = 519). Mean ± SE is shown for both groups.



Wildcats deposited fecal marks on plants with diameters > 26 cm even though plants with diameters of 6-20 cm were the most abundant in the environment (Fig. 12a). However, the wildcats did not exhibit a pattern in the selection of plant height because the heights of the marked plants were similar to those expected if chosen at random in the environment (Fig. 12b).

Fig. 12. Frequency distributions of the (a) diameter and the (b) height of feces-marked plants compared with those of non-marked plants.



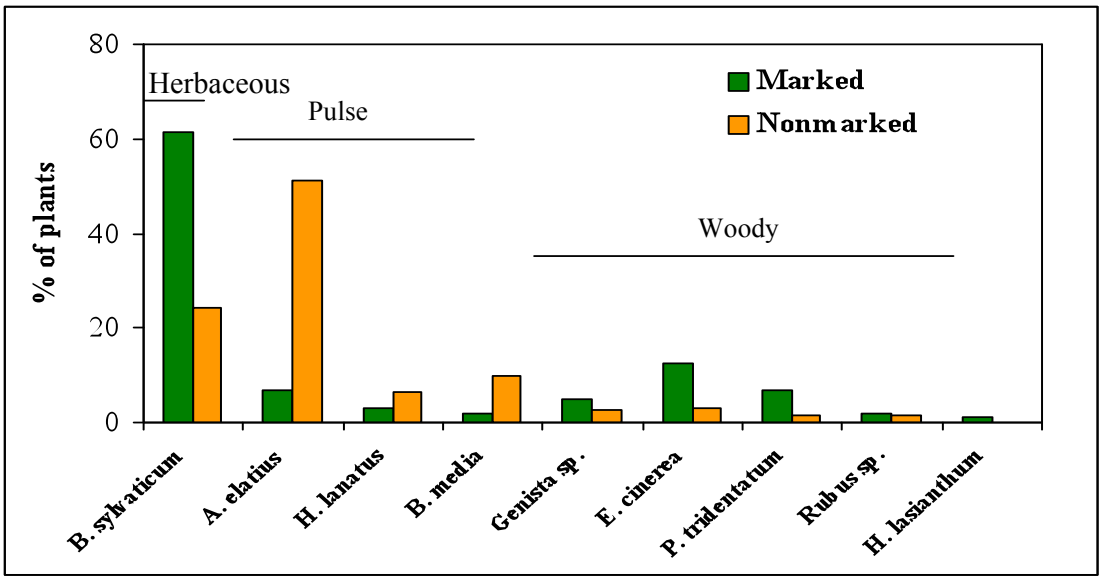


With respect to feces deposition, 66.3%, 22.2%, and 11.5% feces were deposited on herbaceous, woody, and pulse plants, respectively. Both herbaceous and woody plants were more often marked with feces than expected if chosen at random. However, the plants included in the pulse group were used less frequently than that expected by their availability in the environment. These differences in the use of different vegetal groups for depositing

fecal marks, given the environmental availability of these vegetal groups, were statistically significant (χ^2 = 154.15, df. = 2, P = 0.0001, N = 623). The values of Jacobs' selection index indicated that woody and herbaceous plants were neither selected nor rejected depending on their availability in the environment (D = -0.12 and -0.17, respectively), while plants in the pulse group were rejected for depositing fecal marks (D = -0.74).

The differences in the use of different plant species for depositing fecal marks in relation to their environmental availability were also significant (χ^2 = 162.13, df. = 8, P = 0.0001, N = 104). False brome (*Brachypodium sylvaticum*), bell heather (*Erica cinerea*), prickled broom (*Pterospartum tridentatum*), broom (*Genista sp.*), blackberry (*Rubus sp.*), and yellow rock rose (*Halimium lasianthum*) were marked more frequently than expected for their availability, whereas, tall oatgrass (*Arrhenatherum elatius*), quacking grass (*Briza media*), and yorkshire fog (*Holcus lanatus*) were marked at frequencies lower than relative to their environmental availability (Fig. 13).

Fig. 13. Proportion that each plant species was marked with feces by wildcats relative to the proportion of plants of each species at the study site.



Discussion

The results of this study showed that certain characteristics of plants, namely size, vegetal group, and visual conspicuousness, determine their selection as fecal-marking posts by wildcats. Wildcats in the study area selected plants with greater diameters possibly because this characteristic enhances the visual component of the mark as suggested in other carnivore species (Barja 2009). The fact that the studied wildcats deposited their fecal marks on visually conspicuous plants besides choosing plants with greater diameters seems to increase the detection probability of fecal marks by other individuals, including both competitors (reflected in more effective territorial defense) and potential mates. Furthermore, because wildcats defend large territories where constant monitoring is impossible, scent marks must be able to function in their temporary absence and indirectly communicate to intruders of the potential of being discovered by the owner (Richardson 1993). The present results support the economic approach to scent marking proposed by Roberts and Gosling (2001) in which scent marks are placed to maximize their chance of being detected by competitors and potential mates. Territory owners not only deposit scent marks to intercept intruders but also to announce their presence for facilitating their own detection and maximizing the resulting benefits. Thus, many carnivores deposit their scent marks in potential contact zones between territories, including trails and crossroads (Smith et al. 1989, Zub et al. 2003, Barja et al. 2004).

The present results indicate that wildcats do not select plants to deposit fecal marks according to their height because they marked vegetal substrates whose heights were the most abundant in the environment. However, by depositing their fecal signals on plants, wildcats increase the diffusion of the signal, thereby increasing their active field (Alberts 1992); this is supported by the diffusion model of Bossert and Wilson (1963), which states that the parameters of pheromone transmission have been adjusted in the course of evolution to obtain a high degree of efficiency. In this way, the frequent use of tall substrates as marking posts is observed in other carnivore species (Peters and Mech 1975, Barja et al. 2001, Tsegaye et al. 2008, Barja 2009).

Another possible explanation for the preference of plants with greater diameter is that it is used for an animal's self-orientation. In this sense, mammals are generally well equipped to perceive and memorize visual landmarks (Etienne et al. 1999). However, nocturnal species such as the wildcat may depend more on scent marks for orientation than diurnal species. Thus, the deposition of fecal marks on visually conspicuous plants could enhance the presence of visual landmarks or use them as olfactory landmarks to form a cognitive map. Lyall-Watson (1964) suggested that green acouchi (*Myoprocta pratti*) adds odor to specific visual landmarks to familiarize itself with its environment. Likewise, other animals combine visual and olfactory landmarks to navigate (toads: *Bufo bufo*, Sinsch 1987; various monkeys species, Bicca-Marques and Garber 2004) because bimodal sensory input accelerates the acquisition of landmark information (Steck et al. 2011)

Another characteristic that appears to influence the decision making of wildcats to deposit their fecal marks is the vegetal group to which a plant belongs. Our results indicate that herbaceous and woody plants were used more often than expected according to their availability. However, pulse plants were generally rejected for fecal mark deposition. The selection of woody plants may be related to their characteristics (e.g., rough-textured evergreen leaves) that allow the signal to remain for a longer time, maximizing the probability of detection by intruders and potential mates. Substrate texture is an important factor during scent marking in brown bears (*Ursus arctos*) (Clevenger and Purroy 1991) and giant pandas (*Ailuropoda melanoleuca*) (Schaller et al. 1985). Thus, the strong odor of a freshly marked plant might add an olfactory signal to scent marks (Bowyer et al. 1994). In contrast, wildcats may not deposit fecal marks on pulse plants because of their smaller size, which makes them less visually conspicuous, and because they represent sparse foliage, which leaves the mark most exposed to the elements, causing both the scent and visual signal to deteriorate rapidly.

The choice of false brome as the most common plant species on which wildcats deposited fecal marks may be related to its size. In addition, many broad leaves of this plant species help the mark to remain detectable for a longer period. However, further studies are needed to determine which characteristics of plants increase the persistence time of fecal marks of carnivores. Selection of certain plant species by carnivores for depositing their scent

marks has been sparsely documented (grizzly bear, *Ursus arctos* and black bear *Ursus americanus*: Lloyd 1979; Iberian wolf, *Canis lupus*: Barja 2009). Another aspect that may explain the high frequency of fecal marking on false brome is the defense and marking of an important resource. This plant appeared frequently in the diet of wildcats in the study area (Piñeiro and Barja 2011). Cats seem to eat rough grass to regurgitate hairballs or intestinal parasites (Engel 2003). Other mammalian species are reported to have a preference for placing scent marks on plants on which they feed as a method of proclaiming their ownership, including klipspringers (*Oreotragus oreotragus*) (Roberts 1997), common marmosets (*Callithrix jacchus*) (Lázaro-Perea et al. 1999), golden lion tamarins (*Leontopithecus rosalia*) (Miller et al. 2003) and Verreaux's sifaka (*Propithecus verreauxi*) (Lewis 2005).

The results of the present study suggest that the visual components of a vegetal substrate determine whether a wildcat selects a plant to deposit fecal marks. However, wildcats may intentionally select certain plants as visual and olfactory landmarks for spatial orientation alone. Nevertheless, further studies are needed to clarify this. Because of the importance of certain vegetal substrates in the fecal marking behaviour of wildcats and the need to select the most effective locations so that the marks are detected by other congeners, removing these marks while clearing the vegetation may alter the marking behaviour and orientation of wildcats, eliminating the potential marking sites and navigation marks and thus affecting the spacing of the animals.

CAPÍTULO 3.

EVALUATING THE FUNCTION OF WILDCAT FAECAL MARKS IN RELATION TO THE DEFENCE OF FAVORABLE HUNTING AREAS

Abstract

There have been no studies of carnivores that have been specifically designed to examine the function of faecal marks in trophic resource defence, although some chemical communication studies have discussed the function of these marks. The aim of this study was to test the hypothesis that the faecal marks deposited by wildcats (*Felis silvestris*) serve to defend their primary trophic resource, small mammals. Field data were collected over a two year period in a protected area in Northwestern Spain. To determine the small mammals abundance in different habitat types, a seasonal live trapping campaign was undertaken in deciduous forests, mature pine forests and scrublands. In each habitat, we trapped in three widely separated UTM (Universal Transverse Mercator) cells. At the same time that the trapping was being performed, transects were conducted on foot along forest roads in each trapping cell and in one adjacent cell to detect wildcat fresh scats with or without a marking function. The results of the ANCOVA analysis indicated that small mammal abundance and the habitat type were the factors that explained the largest amounts of variation in the frequencies of faecal marking. Also, a significant positive correlation was found between the number of scats with a marking function and the of small mammals abundance. This suggests that wildcats in the park defended favourable hunting areas by depositing faecal marks in substrates and zones that maximise their detectability by congeners. This would allow wildcats to protect their main trophic resource and would reduce intraspecific trophic competition.

Introduction

Studies conducted on mammals have revealed that scent marks have important functions, such as in territory defence (Ahlbom and Jackson 1988, Molteno et al. 1998, Shinn 2002, Broomhall et al. 2003, Gorman and Mills 2009); advertising reproductive condition (Ahlbom and Jackson 1988, Mellen 1993, Molteno et al. 1998, Broomhall et al. 2003); advertising social status (Johnson 1973, Ahlbom and Jackson 1988); identifying individuals, groups and species (Rich and Hurst 1998, Fendt 2006, Müller and Manser 2007); preventing intrasexual competition (Müller and Manser 2007); indicating previously used food patches

(Corbett 1979, Kruuk et al. 1993, Zub et al. 2003); assisting in optimal foraging (Gosling 1982, Sillero-Zubiri and Macdonald 1998); and assisting in resource defence. Animals compete for resources, such as food, mates and shelter (Maher and Lott 1994, Gese 2001), and defend these resources by means such as intraspecific communication by depositing visual and scent marks in their territory (Johnson 1973, Ahlbom and Jackson 1988, Robinson and Delibes 1988, Gosling and MacKay 1990, Sunquist and Sunquist 2002). During intraspecific communication, scent and visual marks are placed in conspicuous places in order to maximise sign detectability (Gosling and Roberts 2001). As a result, many carnivores deposit their faecal marks on conspicuous substrates, above ground level, at crossroads and in latrines (Peters and Mech 1975, Corbett 1979, Robinson and Delibes 1988, Sunquist and Sunquist 2002, Zub et al. 2003, Barja et al. 2004, Barja et al. 2005, Barja 2009, Gorman and Mills 2009).

Scent marking behaviour has been studied in several different felid species: wildcat (Corbett 1979), snow leopard (*Uncia uncia*) (Ahlbom and Jackson 1988), Iberian lynx (*Lynx pardina*) (Robinson and Delibes 1988), ocelot (*Leopardus pardalis*) (Emmons 1988, Shinn 2002), black-footed cat (*Felis nigripes*) (Molteno et al. 1998) and Eurasian lynx (*Lynx lynx*) (Schmidt and Kowalczyk 2006). Nevertheless, there have been no studies on the function of scent marks in the defence of trophic resources in felids. Felids use urine, faeces and secretions of different glands as visual and scent marks that they deposit frequently in obvious places. Deposits are often made in locations with little vegetation, frequently along forest roads and on conspicuous substrates and above ground level to increase the efficiency of the marks (Corbett 1979, Panaman 1981, Gosling 1985, Macdonald 1985, Schmidt and Kowalczyk 2006). The principal form of intraspecific communication among wildcats, as in other felids, is also based on the placement of visual and scent marks in their environment. Animals delimit their territory by depositing marks on trails, at crossroads, and on raised and conspicuous substrates (Peters and Mech 1975, Corbett 1979, Sunquist and Sunquist 2002, Zub et al. 2003). Wildcats use urine, faeces and different specialised glandular secretions (interdigital, anal and facial glands) as visual and scent marks (Kleiman and Eisenberg 1973, Corbett 1979, Sunquist and Sunquist 2002).

The wildcat is a solitary and territorial carnivore in which intraspecific contact is mainly limited to the breeding season (Sunquist and Sunquist 2002). The home range size of this species varies between 4,828 and 5,206 ha for males and between 608 and 727 ha for

females. Males typically have home ranges that overlap those of several females (Urra 2003). Both sexes share a territory, associate only during the mating season, during which chemical communication is very important. Studies conducted on several small felids, including the wildcat, have shown that reproductive cats deposited marks more frequently than non-reproductive cats (Mellen 1993). Similarly, Corbett (1979) found that the marking rate of an adult male increase when a transient wildcat passes through his range, and that adults cats had higher marking rates than young cats.

For several felid species, territoriality seems to be related to the availability of key resources (Azevedo and Murray 2007). Therefore, scent and visual marks (e.g. faeces, urine and glandular secretions) are deposited to identify territory edges and to defend trophic resources from potential competitors (Ahlbom and Jackson 1988). Thus, competition for food should be lower in areas with a high availability of resources and when defence costs are lower than the benefits of having exclusive use of a resource (Azevedo and Murray 2007). Visual and scent marks deposited by resource holders provide a means of reducing the cost of resource defence if the recipients of this scent mark decide to avoid the conflict (Erlinge et al. 1982, Richardson 1993, Maher and Lott 1995, Rich and Hurst 1998, Gosling and Roberts 2001). The resident individuals are generally prepared to defend their resources. Thus, intruders can identify residents using these marks and can calculate the costs and benefits of competing for the defended resources (Gosling and MacKay 1990, Gosling and Roberts 2001). Some studies have also indicated that the deposition of urine marks serves to minimise the forage time in mammals such as red foxes (*Vulpes vulpes*) (Henry 1977), Iberian wolves (*Canis lupus*) (Harrington 1981), coypuses (*Myocastor coypus*) (Gosling and Wright 1994), coyotes (*Canis latrans*) (Gese and Ruff 1997) and common marmosets (*Callithrix jacchus*) (Lázaro-Perea et al. 1999).

This study was designed to test the hypothesis that the faecal marks deposited by wildcats serve to defend their trophic resources. Therefore, one could predict that: 1) wildcats will deposit a greater number of faecal marks in habitats within their territory that contain a higher abundance of their principal prey (favourable hunting areas) to protect their main trophic resources and to reduce intraspecific competition; and 2) faecal marks that function to defend trophic resources should be deposited in substrates and zones that increase their detection.

Material and methods

Abundance of small mammals

Previous studies conducted in the study area indicated that small mammals constitute the principal prey of wildcats and that their abundance changes with the habitat type and zone of the park (Piñeiro and Barja, unpublished data). Therefore, to determine the abundance of small mammals (the main trophic resource), from August 2005 to June 2007, seasonal live trapping were undertaken during 9 days in the most representative habitats of the study area (deciduous forest, mature pine and scrubland; total effort: 4,725 traps-night). In each habitat, we selected three different UTM (Universal Transverse Mercator) cells that were separated at minimum of 3 km to conduct live trapping. The number of small mammals obtained for each of the 8 trapped cells was then extrapolated to each adjacent UTM cell, choosing the nearest and with habitat characteristic more similar to each trapped cell. Therefore, during this study we trapped 8 different cells and we extrapolated the number of small mammals to 8 adjacent cells. In each cell, we placed a grid containing 25 Sherman traps, separated by 10 m, which covered an area of 0.25 ha at each sampling point. The traps were left open 24h for three consecutive nights and bread soaked in oil was used as bait. However, to minimise the time that small mammals were in the traps and potential vulnerability to predators, traps were monitored at least every 12 h (sunrise and sunset) (Gurnell and Flowerdew 1994, Powell and Proulx 2003). During study bedding in live-traps were used to reduce mortalities; we placed raw wool with a natural lanolin because is an excellent insulator that repels water. Also, traps were set under cover of shrubs or dense herbs to conceal them from harassment by predators and to provide some thermal insulation (Gurnell and Flowerdew 1994, Powell and Proulx 2003). Nevertheless, no evidence of predators approaching traps was recorded during the study. To allow the identification of each individual for later recapture and to thus avoid pseudoreplication in the abundance data, and to avoid that the animal was more conspicuous to predators, a minimum amount of non-toxic, waterproof, colored spray paint were applied to the chest, paws or tail root of all individuals during their first capture. After the handling, the small mammals were released at the point of capture.

The number of pregnant or lactating females caught was very low (3.4%) and only 2.6% small mammals died as a result of trapping conducted for this study. We followed

ASAB/ABS guidelines for the treatment of animals in behavioural research and teachers (Sherwin 2006). Research was undertaken under permit from the Xunta Galicia wildlife authority (letter of 18/04/05, 18/09/06, 13/07/07).

Detection of faecal marks

To detect the scats we established transects along forest roads because wildcats, as other medium size carnivores (red fox, stone marten and European pine marten), often use roads for travelling and frequently defecate on the roads as a means of visual and scent marking (Corbett 1979, Barja 2005a). From August 2005 to June 2007, transects along forest roads were conducted on foot in the trapped cells and on adjacent cells to locate wildcat fresh scats and to record the number of scats deposited with or without a presumed marking function. A scat was considered to have a marking function if its location was described by at least two of the following characteristics: 1) on a conspicuous substrate; 2) above ground level; 3) at a crossroad; or 4) in a latrine (i.e., within an accumulation of two or more scats; Barja et al. 2005). Substrates were classified as inconspicuous or conspicuous, the latter being referring to substrates that could be easily observed within a 1 m radius of the scat by a human observer. All other scats were determined to be as inconspicuous (Barja et al. 2004, 2005, Barja 2009).

Wildcat scats were differentiated from those of other medium carnivores present in the study area by their morphological characteristics (size and shape). The scats of wildcats, domestic cats and their hybrids are very similar and are difficult to differentiate. However, the nearest human population was 7 km to the south of the study area; the presence of domestic cats would, therefore, at the very least be greatly reduced. During this study, 24 cats were observed and another 8 were photographed using camera traps; all cats showed typical wildcat external morphology (Stahl and Leger 1992, Kitchener 1995, Sunquist and Sunquist 2002). None of the faeces detected along the transects were buried; indeed, they often had a marking function and were deposited in prominent locations. Wildcats are reported to show this behaviour (Corbett 1979, Barja and Bárcena 2005) while domestic cats tend to bury their faeces in areas where both domestic and wildcats occur (Corbett 1979, Schauenberg 1981). This difference in behaviour can therefore be used to differentiate the faeces of these cats. In addition, recent studies conducted in different European populations on interbreeding between wildcats and feral domestic cats indicate that hybridisation is a local problem (Randi et al.

2001, Pierpaoli et al. 2003, Lecis et al. 2006, Oliveira et al. 2008b). A study conducted in Spain showed an introgression rate of less than four percent (Ruiz-García et al. 2001). The presence of domestic cats and hybrids in the study area was therefore not very likely, confirming the origin of the scats studied.

Data analyses

The length of roads surveyed in the cells was different ranged between 1.6 km and 4.0 km, therefore, we used a kilometric abundance index that was produced by dividing the number of scats with or without a marking function that were detected by the number of kilometres surveyed in each cell.

As the data were not normally distributed, we performed a logarithmic transformation of the quantitative variables prior to analysis to ensure normality (Kolmogorov-Smirnov test) and homoscedacity (Levene's test). We used a mixed general linear model (ANCOVA) to test whether the frequency of faecal marking varied in relation to habitat type (fixed factor). We included season as random factor and small mammal abundance as a covariate. The months of the year were pooled into seasons: April-June (spring), July-September (summer), October-December (autumn), January-March (winter). One-way analyses of variance (ANOVA) were used to determine the effects of habitat type on the frequency of faecal marking and on the abundance of small mammals. A Pearson's correlation was used to test the relationship between the number of faecal marks and the abundance of small mammals. The results are given as means ± standard error (SE). The significance was set at $P < 0.05$. All analyses were performed with SPSS v.15.0 software for Windows (SPSS Inc, Chicago, IL, U.S.A.).

Results

During the study, a total of 194 wildcat scats were observed (51.5 % with a presumed marking function and 48.5% without a marking function) and a total of 232 small mammals were captured. The mean number of faecal marks was significantly higher in scrubland than in deciduous forest and mature pine forest (Fig. 14; ANOVA: $F_{2,97} = 11.153$, $P = 0.0001$; a Games-Howell´s test, $P < 0.05$, was used to compare mature pine with scrubland and to compare deciduous forest with scrubland). The abundance of small mammals also differed

significantly between the three habitat types (Fig. 14; ANOVA: $F_{2,191} = 740.668$, $P = 0.0001$; Games-Howell´s test, $P < 0.05$ for all treatments).

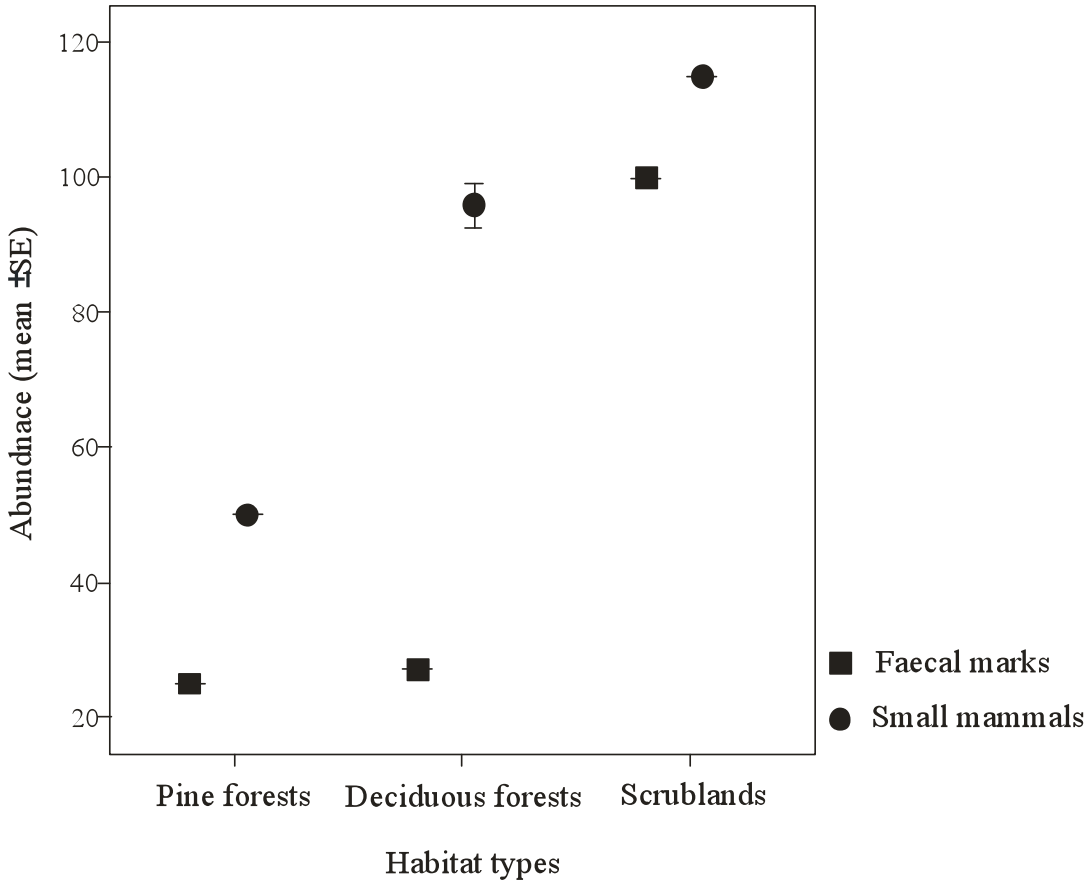


Fig. 14. Variation in the number (mean ± SE) of faecal marks and small mammals in relation to habitat type (pine forest, deciduous forest and scrubland).

The results of the ANCOVA analysis (Table 7) indicated that the abundance of small mammals in the trapped cells and, to a lesser extent, the habitat determined the largest amount of variation in the frequencies of faecal marking. Nevertheless, the season and the interaction between habitat and season did not influence the deposition of the faecal marks (Table 7).

Table 7. Results of the ANCOVA examining the effects of the abundance of small mammals, habitat and season on the abundance of faecal marks deposited by wildcats.

Factors	*F*	df	*P*
Abundance of small mammals	5.68	1	0.019
Habitat type	5.22	2	0.048
Season	1.49	3	0.310
Habitat type * Season	1.197	5	0.317

A significant positive correlation was found between the number of scats with a marking function and the abundance of small mammals in the cells where trapping was conducted (Pearson correlation: $r = 0.263$, $N = 100$, $P < 0.05$). On the other hand, the number of scats without a marking function and the abundance of small mammals were not significantly related (Pearson correlation: $r = 0.157$, $N = 94$, $P > 0.05$).

Discussion

The results of this study showed that the abundance of principal prey and habitat type were the factors that determined the faecal marking behaviour in the wildcat. Wildcats deposited a greater number of faecal marks in those habitats with a higher abundance of small mammals. Previous studies conducted in the study area showed that the wildcat diet was comprised mainly of small mammals during all seasons (Piñeiro and Barja, unpublished data). The results obtained in this study supported the hypothesis suggested by Corbett (1979) that wildcats deposit a higher number of faecal marks in resting areas and in the principal hunting areas. The wildcat is a solitary species that uses scent marking to defend its territories from potential competitors (Kitchener 1991) and, therefore, to defend its critical resources (food, mates and refuges; Gese 2001).

The wildcats in the study area deposited a greater number of faecal marks in scrubland areas, the habitat with a higher abundance of their principal prey (small mammals). These results were consistent with the economic approach to scent marking proposed by Gosling

(1981), which predicted a greater number of scent marks in locations where the preferred prey is abundant. However, these results appeared to contradict those of Sunquist and Sunquist (2002) who supported the idea that felids show a marked territorial behaviour in areas where food is scarce. On the other hand, the wildcats in the study area deposited a greater number of faecal marks on conspicuous substrates and at strategic sites within their territory (crossroads). This increased the efficiency of the scent marks and the probability of detection by other individuals, as indicated in other species of carnivores (Eaton 1970b, Macdonald 1985, Emmons 1988, Robinson and Delibes 1988, Broomhall et al. 2003, Barja et al. 2004, Barja 2009). These results also supported the second prediction of the economic approach to scent marking (Gosling 1981), which indicates that scent marks should be placed on substrates that increase their efficiency and in zones of the territory where the probability of detection by competitors is higher.

The high number of faecal marks deposited along forest roads that cross scrubland zones in comparison with those that cross other habitat types (deciduous forest and pine forest), appears to be related to the higher abundance of small mammals in scrublands, which offer more refuges for these prey species. On the other hand, the lower number of faecal marks detected in the pine forest appears to be related to the low prey availability in that habitat indicated by studies of habitat selection in wildcats in Europe (Corbett 1979, Easterbee et al. 1991). These studies indicated that wildcats rarely use this habitat type due to the low abundance of prey caused by a lack of refuge for prey species. Finally, the small number of faecal marks deposited by the wildcat in the deciduous forests of the study area, where small mammals were abundant, might be related to the competition for this trophic resource with another medium sized carnivore present in the study area, the pine marten. Deciduous forests are the preferred habitat of this mustelid in the study area (Barja 2005a) and the small mammals also constitute its principal prey (Rosellini et al. 2008). Consequently, wildcats exploit and defend their primary trophic resources in those habitats where this prey species is abundant and in locations where there is less interspecific competition. In addition, this fact was supported by recent studies conducted on wildcat habitat selection in the Iberian Peninsula. These studies indicate that the felid is not a strictly forest species; wildcats prefer open fields made up of scrublands and pastures (Lozano et al. 2007). Therefore, it is possible to consider scrublands as a key element of the environment of that species. The importance of

the principal prey on habitat selection and home range size has been documented for many species of felid (Rabinowitz and Nottingham 1986, Ahlbom and Jackson 1988, Jedrzejewski et al. 2002, Broomhall et al. 2003, Manfredi et al. 2006, Lozano et al. 2006, Lozano et al. 2007).

The high number of faecal marks deposited by the wildcat in favourable hunting areas can also help to optimise the food search, a basic process assuring the survival of individuals. This scent marking strategy is a common pattern observed in many other mammals that minimises the forage time, increases the efficiency of foraging in this way (as observed in the following species: red fox: Henry 1977; Iberian wolf: Harrington 1981; coyote: Gese and Ruff 1997; and common marmoset: Lázaro-Perea et al. 1999). Thus, resource holders can use scent marks to advertise to other inter- and intra-specific individuals exploiting the same food resource that the resources at that location have been and are being exploited. Thus, the time required for a food search decreases and the effort is centred on favourable hunting areas which can be located using their spatial memory, as is observed in other carnivores (Macdonald 1976), but also using the large number of faecal marks deposited in these areas. Also, the faecal marks deposited by wildcats, as food resource holders, might provide a way of advertising its presence to potential intruders, thus reducing the costs of resource defence (Gosling and Mackay 1990, Azevedo and Murray 2007). In addition, these marks may present a means of avoiding agonistic encounters between competitors (Gosling and Mackay 1990, Sillero-Zubiri and Macdonald 1998, Zub et al. 2003, Azevedo and Murray 2007) because intruders will avoid marked areas (Gosling and Mackay 1990).

The results of this study indicated that season is not an important factor determining the number of faecal marks deposited by the wildcats. Faecal marking behaviour is of vital importance throughout the year. During the non-reproductive period, resident wildcats primarily appear to defend their food resources, attaching more value than to their partners (Sliwa 1996). However, during the reproductive period, territorial marking seems to be more important to intrasexual competition (Ralls 1971, Johnson 1973, Macdonald 1985).

CAPÍTULO 4.

EFFECTS OF TOURIST PRESSURE ON REPRODUCTION AND PHYSIOLOGICAL STRESS RESPONSE IN WILDCATS: MANAGEMENT IMPLICATIONS FOR SPECIES CONSERVATION

Abstract

Ecotourism and human recreational activities are increasing and can have a significant impact on fauna. The analysis of faecal glucocorticoid levels is a non-invasive method of measuring physiological stress responses of wildlife to various factors (i.e., human disturbances). The aim of this study was to examine the effects of tourist pressure and reproduction on physiological stress responses in wildcats. The study was conducted from May 2005 to June 2009 at Montes do Invernadeiro Natural Park (NW Spain). Natural Park is divided into three areas depending on the level of protection: restricted public-use, restricted area and integral reservation. To detect fresh scats of several wildcats, we conducted transects on foot along forest roads in the three areas of the park. The number of visitors per day was recorded. An enzyme immunoassay technique was used to quantify cortisol and sex hormones in faeces. A total of 110 fresh faecal samples were collected. The general linear model indicated that park area and faecal progesterone levels were the factors that explained the variation in the faecal glucocorticoid levels. Cortisol levels were higher in park areas where tourist pressure was more intense (restricted public-use area). Faecal cortisol levels were also higher when progesterone concentrations were more elevated (spring) and during the young dispersal period (autumn). The results demonstrate negative effects of tourism on wildcats at the physiological level. Therefore, we recommend that some areas be maintained free of visitor impact and that visitor number be controlled during the animals' sensitive periods (gestation and young dispersion).

Introduction

The reduction in biodiversity values that has occurred in recent decades has its origin in a series of phenomena linked to human activities. These include global warming as well as fragmentation, loss and transformation of habitats, all of which have a strong impact on the environment (Tilman et al. 2002, Benton et al. 2003, Thomas et al. 2004, Parmesan 2006, Shepherd and Whittington 2006, Theuerkauf et al. 2007). The effects of human activities on animal populations are widely discussed by biologists, politicians, industry and the public in general. However, there is no general consensus on the best way to mitigate these effects.

The field of conservation physiology, which has arisen over the past few decades, is a discipline that can help improve the conservation of threatened and endangered species through endocrine approaches. The levels of glucocorticoid 'stress hormones' in different mammalian species have been used as indicators of human disturbances caused by environmental changes such as sports activities (e.g. Iberian wolves, *Canis lupus* and elks, *Cervus elaphus*: Creel et al. 2002), human presence (e.g. bears, *Ursus arctos*: Von der Ohe et al. 2004), tourist pressure (e.g. European pine marten, *Martes martes*: Barja et al. 2007; and spotted hyenas, *Crocuta crocuta*: Van Meter et al. 2009) and pastoralist activity (e.g. spotted hyenas, *Crocuta crocuta*: Van Meter et al. 2009).

In a number of studies, it has also been shown that physiological stress levels in wild mammalian species appear to influence in sex steroid hormones levels; e.g. baboons (*Papio anubis*) (Sapolsky 1986), free-ranging cheetahs (*Acinonyx jubatus*) (Terio et al. 2004). For both males and females, reproduction is at risk due to stress, but in most species the female appears most vulnerable to behavioural stress (Moberg 1985). On the other hand, Sapolsky (1986) showed a relationship between elevated testosterone levels and social stress caused by the social factors (e.g. group size, social status). In dominant males, an increase in testosterone levels probably confers a reproductive advantage by accentuating aggressive tendencies during competition over mates.

Animals can survive in changing external environments due to the flexible stability of their internal environments (Greenberg et al. 2002). Physiological stress can be defined as an adaptive response of an organism to a demand made by its environment (Möstl and Palme 2002). The stress response includes a series of physiological and behavioural changes that occur in order to restore homeostasis in an organism (Schwarzenberger et al. 2000, Möstl and Palme 2002). In animals, physiological stress responses to disturbances of the environment are adaptive in the short term; however, when glucocorticoid levels remains elevated for longer periods of time ('chronic stress'), a wide range of negative consequences can occur, including immune and reproductive suppression (Sapolsky 1992, Sapolsky et al. 2000).

Glucocorticoid levels as indicators of stress can be quantified in plasma, urine, saliva and faecal samples. Faecal glucocorticoid quantification provides a non-invasive method, avoiding the additional stress that can be caused by animal capture, handling, sedation and/or transport (Graham and Brown 1996, Brown and Wildt 1997, Dehnhard et al. 2001).

Some felid species, including wildcats (*Felis silvestris*), face conservation problems as a result of destruction and alteration of their habitat caused by human intervention. The wildcat is listed on Appendix II of CITES, which aim is to ensure that international trade in specimens of wild animals and plants does not threaten the survival of the species in the wild. Also, the specie is listed in Annex IV of the EU Habitats, which aims to protect the wild plants, animals and habitats that make up our diverse natural environment. Furthermore, the wildcat is included in Species Directive and Appendix II of the Bern Convention. The aims of this Convention are to conserve wild flora and fauna and their natural habitats, especially those species and habitats whose conservation requires the co-operation of several States. Finally, the wildcat is classified as 'least concern' at the national level in many European range states (IUCN 2011). The main threats that affect the species in Europe include alteration of its habitat, hybridisation and exchange of diseases with feral cats and persecution (Stahl and Artois 1991, Kitchener 1995), Worldwide, ecotourism and recreational activities are growing, especially in protected natural areas (Balmford et al. 2009). For the conservation of the wildcat and other species, it is therefore important to know the effects these increased recreational activities have on animals at the physiological level. Thus, the knowledge of these effects on the fauna could be useful in the management of human disturbances, thereby reducing the negative effects on the conservation of the species.

Most studies of how human disturbances affect felids have been based on behavioural changes (Van Dyke et al. 1986, Gagliuso 1992, Kerley et al. 2002, Ngoprasert et al. 2007, Jerosch et al. 2010); only a few studies performed on animals in captivity have used physiological parameters to assess the sensitivity of felids to human presence (Terio et al. 2004, Montanha et al. 2009). Therefore, it is important to conduct field studies which integrate the behavioural analysis and other non-invasive physiological evaluations to provide a meaningful measure of physiological stress.

Wildcats are seasonal breeders (Kitchener 1995). They are sexually mature at about ten months and females may conceive on their first estrus. Males and females are mostly come into oestrus and commence spermatogenesis between late December and the beginning of August (Kitchener 1995), with mating most frequent between January and March (Nowell and Jackson 1996). Gestation lasts two months and takes place between March and May. Litter sizes range from 1 to 8 (Stahl and Leger 1992). Kittens become independent at about 5

months, just before the winter, and can travel several miles to establish their own home ranges (Corbett 1979, Kitchener 1995).

The aim of this study was to determine whether increased physiological stress levels in wildcats was a response to the level of tourism allowed within different zones within the park and/or a response to the seasonal reproductive state of wildcats. If these factors increase physiological stress in this species, it is possible to predict that 1) faecal cortisol metabolite levels in wildcats will be higher in the zone where tourism is least restricted and when the seasonal level of tourism is highest and 2) wildcats will show higher faecal cortisol metabolite levels during the animals' most sensitive reproductive period.

Materials and methods

Faecal sample collection

Fresh faecal samples were collected every two months from May 2005 to June 2009. We surveyed on foot and collected fresh wildcat scats from forest road strips, each 300 m x 7 m. Survey strips were separated by a minimum distance of 700 m. The strips sampled were selected based on the results obtained by Barja and Bárcena (2005) in a previous work on wildcat's habitat selection made in the study area. This study indicated that wildcats show a preference for areas that include valleys with deciduous forests and watercourses. Therefore, we established strips in forest roads that cross zones that had these characteristics.The total number of kilometers surveyed for each zone was as follows: high visitation (130.8 km), low visitation (67.1 km) and no visitation (113.3 km).

The wildcat, like other medium-sized carnivores, often uses roads for travelling and frequently defecates on them as a means of visual-scent marking (Corbett 1979, Barja 2005b). To detect the scats we surveyed the established transects cada 24 h, thus increasing the likelihood of collecting fresh faecal samples. Wildcat scats were differentiated from those of other carnivores present in the park (red fox [*Vulpes vulpes*], pine marten, weasels [*Mustela nivalis*], stoats [*Mustela erminea*] and Iberian wolf) by their size and shape. The wildcat scats are long, cylindrical and thick (with a length of 10-20 cm and a diameter of 1.4-3.0 cm), with contiguous fragments that fit perfectly, because the concave end of one coincides with the

beginning of the next convex fragment (Corbett 1979). Samples of fresh faeces used to quantify sex hormone and glucocorticoid levels were collected only between sunrise and three hours thereafter to assure that exposure to environmental conditions and microbial action was minimized (Millspaugh and Washburn 2003).

In cougars (*Puma concolor*), faecal glucocorticoid levels increased significantly 24 h after exposure to a stressor (Bonier et al. 2004). Additionally, in African wild dogs (*Lycaon pictus*), faecal corticosterone increased within 24 h after adrenocorticotropic hormone (ACTH) injection (Monfort et al. 1998). Based on these results, for purposes of data analysis we correlated the number of visitors that had been allowed into each zone of the park on the previous day (this number was recorded daily by the gamekeepers) with the dates of collection of individual faecal samples. We collected all samples from the fine end of the scat to standardise sampling procedures. Fresh faeces differed from old ones by the presence of a moist layer of mucus, a strong smell and no signs of dehydration (Liu et al. 2006, Barja et al. 2007). Approximately 10 g of sample was collected from each fresh scat by means of a gloved hand. All samples were frozen at –20ºC for subsequent laboratory analysis.

For each fresh faecal sample, the following data were recorded: date of collection, UTM cell and zone of the park where the sample was collected.

DNA extraction, specific origin and individual genotyping

Specific identification through molecular analysis was conducted to demonstrate the specific origin (from wildcats rather than from feral cats or hybrids) of the faecal samples and the reliability of the data obtained. To obtain the samples, we surveyed on foot in 25 UTM cells of 1 km^2. Individual genotyping was carried out to determine the minimum number of wildcat individuals from which the faecal samples originated. To perform specific identification and individual genotyping, we collected a total of 26 fresh faecal samples spread evenly throughout the study area.

DNA extraction of faecal samples was performed using salting-out and phenol-chloroform extraction (Sambrook et al. 1989). We assessed individual multilocus genotypes using 12 neutral unlinked microsatellites that were formerly isolated and characterised in domestic cat, *Felis silvestris catus* (Oliveira et al. 2008a). Polymerase chain reaction (PCR)

amplification of individual microsatellites followed Randi et al. (2001). Allele frequencies, standard diversity indices and observed (HO) and expected (HE) heterozygosities for each locus and population were calculated using GENETIX 4.05 (Belkhir et al. 1996-2004).

Quantification of faecal glucocorticoids and sex hormones

The levels of cortisol metabolites, testosterone (T), progesterone (P) and estradiol (E) in the faecal samples were analysed. Sex steroid hormones were quantified to determine the effect of the reproductive status of individuals on physiological stress levels. Quantification of faecal glucocorticoids has been used as an indicator of physiological stress in other felid species (domestic cat: Graham and Brown 1996; clouded leopard, *Neofelis nebulosa*: Wielebnowski et al. 2002). All hormones were extracted using previously established methods for similar carnivore species (Brown et al. 1994, 1996, Young et al. 2004, Barja et al. 2007). The efficiency of extraction was tested by the addition of radiolabeled hormones (3H-cortisol, 3H-testosterone, 3H-progesterone and 3H-estradiol, 4000-8000 dpm, ICN, California, USA) to a parallel set of faecal samples prior to extraction.

Sex steroid hormone (SSH) and cortisol metabolite concentrations were determined from faecal extracts by an enzyme immunoassay. Hormone concentrations were calculated by means of software developed for this technique (ELISA-AID, Eurogenetic, Belgium). Standard dose-response curves were constructed by plotting the binding percentage (B/Bo×100) against the standard hormone concentrations added.

The mean recovery percentages from faecal extracts (95% for high concentrations and 98% for low concentrations) were very similar for all hormones. Parallel displacement curves were obtained for each hormone by comparing serial dilutions of pooled faecal extracts with the standard curves. The results showed that both curves were parallel in all cases. Intra- and inter-assay coefficients of variation were calculated by assaying ten replicates of a pooled faecal sample in the same assay and ten replicates of the same sample in ten consecutive assays. The percentages recovered for high and low concentrations were, respectively, as follows: cortisol metabolites, 4.5% and 7.5%; testosterone, 4.5% and 7.5%; progesterone, 4.9% and 9.1%; estradiol, 5.9% and 8.9%. For each sample, SSH and cortisol metabolite concentrations were expressed as ng/g of dry faeces.

Data analysis

Because we found that the variables were not normally distributed (Shapiro-Wilk test), we log-transformed the quantitative variables (cortisol, testosterone, estradiol and progesterone levels, and the number of visitors). We used a generalized linear model to test whether the factor (park zone) and the covariates (sex hormone levels and number of visitors) influence on faecal cortisol metabolite levels (response variable). The Akaike Information Criterion (AIC) (Burnham and Anderson 2002) was used to select the most parsimonious models. In order to select the best GLM models, the Akaike weights of each model was estimated following procedures by Burnham and Anderson (2002). The variables with the highest weight ($\Sigma\omega_m$ = 0.95) being more important relative to the others. Selection of candidate models followed the rule in which those where $\Delta i \leq 2$ have substantial empirical support (Burnham and Anderson 2002). Faecal sex hormone and cortisol metabolite concentrations are given as the mean ± SE. A *P* value < 0.05 was considered statistically significant. All statistical analyses were carried out using SPSS version 15.0 for Windows (SPSS Inc, Chicago, Illinois, USA).

Results

Sample collection and genetic analysis

We collected 110 samples from fresh scats of wildcats. The genetic analysis indicated that all of the analysed samples came from wildcats; no samples were from domestic cats, feral cats or hybrids. A total of 16 different wildcat genotypes were identified.

Stress levels in relation to park zone and season

The best models (ΔAIC<2) indicated that the variables park zone (ω_m= 1), progesterone levels (ω_m= 1) and number of visitors (ω_m= 0.836) were the only factors that explained variations in faecal cortisol metabolite levels (Table 8).

Table 8. Ranking of the best models ($\Sigma\omega_m$=95%) explaining the variation in faecal corticosterone levels. The table shows number of parameters (K), AICc, AICc differences (Δ_i), Akaike weights (ω_m), and the rank of the models. PL: $\log_{10}$Progesterone levels, EL: $\log_{10}$Estradiol levels, TL: $\log_{10}$Testosterone levels, NV: number of visitors, ZP: park sone.

Model	K	AICc	Δ_i AICc	ω_m	Rank
PL+NV+ZP	4	152.13	0.00	0.399	1
EL+PL+NV+PZ	5	153.07	0.94	0.249	2
PL+TL+NV+PZ	5	153.64	1.51	0.187	3
PL+PZ	3	153.91	1.77	0.164	4

Faecal cortisol metabolite concentrations were higher in forest roads which cross zones of the park where tourism pressure was more intense than in zones with low visitation and no visitation (Fig. 15).

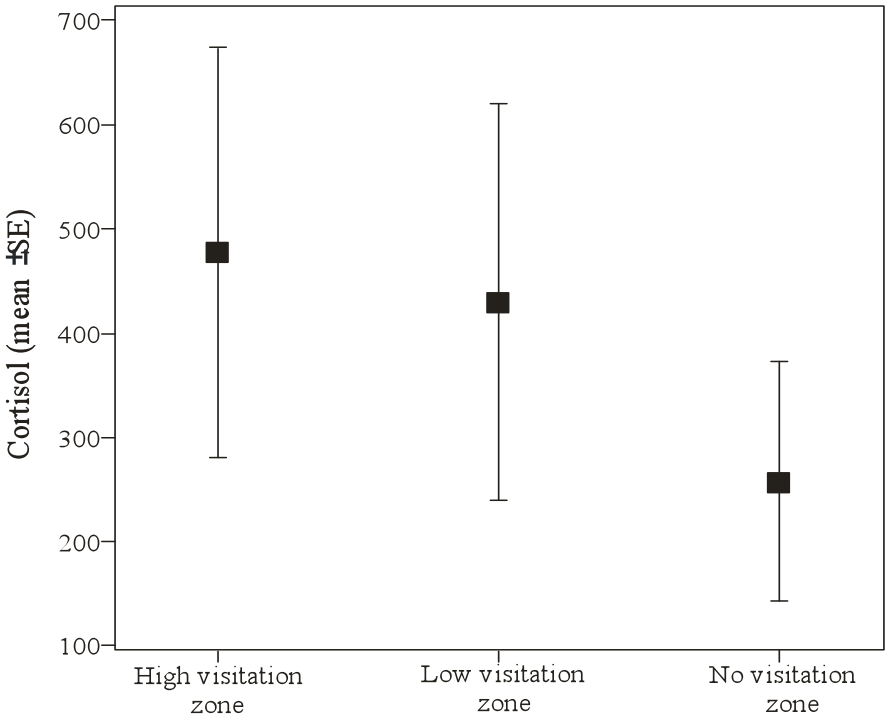


Fig. 15. Comparison of concentrations of faecal cortisol metabolites (mean ± SE) according to tourist pressure in different zones of the Natural Park.

The mean levels of faecal cortisol metabolites were higher in spring and autumn than in winter and summer (Fig. 16). The mean number of visitors allowed into the park was significantly higher in summer and spring than in autumn and winter (Fig. 16).

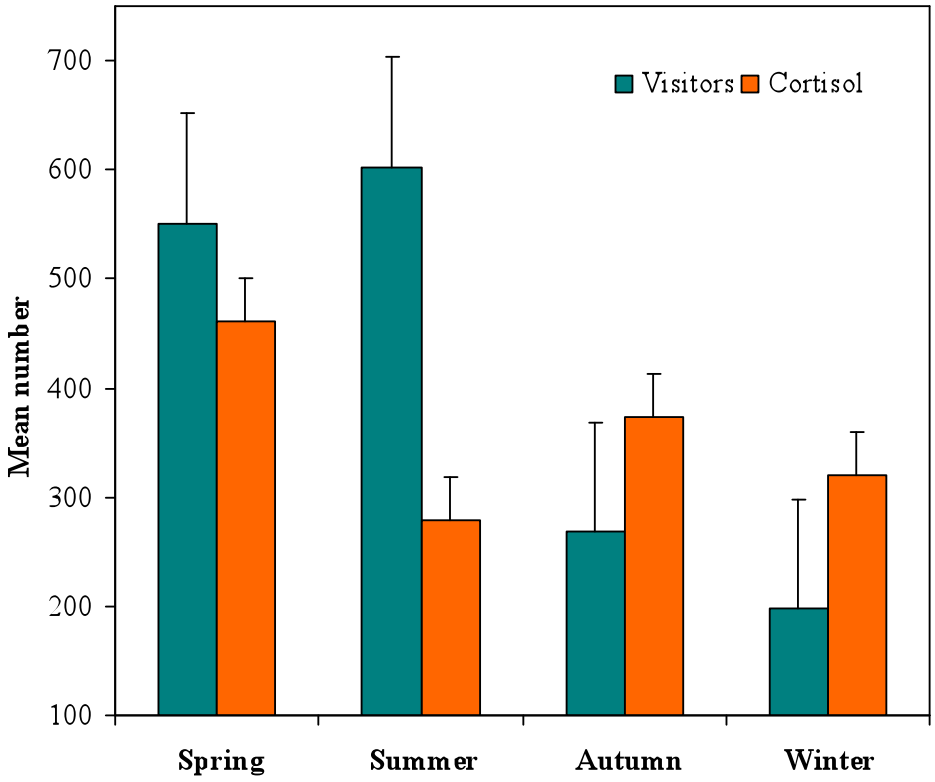


Fig. 16. Mean levels of cortisol metabolites and mean number of visitors per season. Data are given as the mean ± SE.

Sex hormone concentrations in relation to physiological stress levels

Faecal estradiol and progesterone mean concentrations were significantly higher in spring and summer than in winter and autumn (Fig. 17). On the other hand, faecal testosterone mean concentrations were higher in summer and spring than in winter and autumn (Fig. 17).

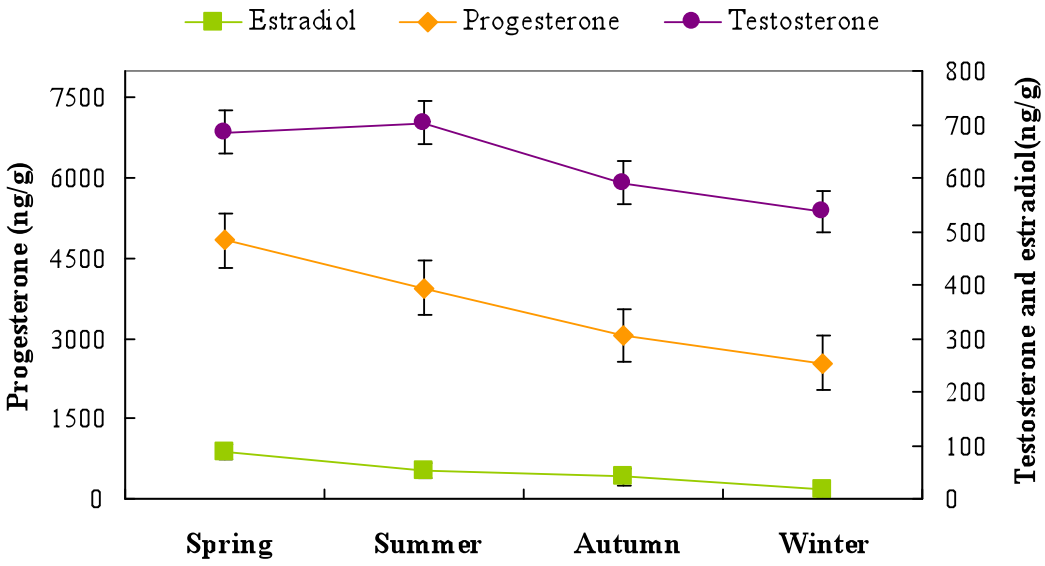


Fig. 17. Mean levels of sex steroid hormones (testosterone, progesterone and estradiol) per season. Data are given as the mean ± SE.

Discussion

The results of the present study indicate that tourist pressure and reproductive period are stressful agents for the wildcat. Wildcat faecal cortisol metabolite levels were elevated in forest roads which cross zones of the natural park where the number of visitors was higher (high visitation zone and, to a lesser extent, low visitation zone). These results concur with those of other studies, in which increases in animals' physiological stress responses to human disturbances such as snowmobile use (Iberian wolf and elk: Creel et al. 2002), ski tourism (capercaillie, *Tetrao urogallus*: Thiel et al. 2008) and human presence (pine marten: Barja et al. 2007; Iberian wolf: Eggermann 2009) were observed. The abundance of wildcats and the abundance of competitors does not seem to be contributing to increase of the cortisol metabolite levels in wildcats in high visitation zone because a previous study conducted in the same study area involving wildcat and other species of medium-sized carnivores (pine marten and red fox) (Barja and Bárcena 2005) indicated that the three species were more abundant in

the no visitation zone (integral reserve) (Barja and Bárcena 2005), possibly due to the higher availability of shelter and prey (small mammals) (Piñeiro and Barja, unpublished data). Therefore, this does not seem to be a plausible alternative explanation for the lower cortisol metabolite levels recorded.

The high levels of faecal glucocorticoids exhibited by the wildcat population in forest roads which cross the high visitation zone indicate that exposure to humans on foot has a negative impact on this species at the physiological level. In wild spotted hyenas, faecal glucocorticoids also increase with exposure to humans on foot but not with exposure to tourists in vehicles (Van Meter et al. 2009). These results should be considered by the managers of protected natural areas in making decisions about the degree of interaction that should be allowed between wildlife and humans. Nevertheless, it is necessary to emphasise the need for more studies on the effect of recreational activities on physiological condition in this and other mammalian species to know which species are vulnerable and to what human disturbances (i.e., habitat quality, sports activities and recreational activities). The results obtained to date indicate that in some species faecal glucocorticoid levels positively correlate with human activities (Creel et al. 2002, Barja et al. 2007, Thiel et al. 2008) but that in Alaskan brown bears (*Ursus arctos horribilis*) there is no relationship between human activity and stress response (Von der Ohe et al. 2004).

With respect to temporal factors, the elevation in faecal cortisol metabolite levels seems to be related to some aspects of the wildcat's reproduction. Thus, faecal cortisol metabolite levels were higher when progesterone concentrations were high (spring) and in autumn during the gestation period of females and the dispersion of the young. In felids, progesterone levels increase after copulation (Graham et al. 1995), helping embryo implantation (Verhage et al. 1976), and reach basal values after parturition (Brown et al. 1994, Graham et al. 1995). In spring, when females were pregnant and physiological stress levels were high, the number of visitors who came to the park was, together with the summer, the highest of the year. This increase of the number of visitors during a sensitive period for the species could seriously affect its reproductive success, as it was observed in other mammals (Arck et al. 1995, Nepomnaschy et al. 2006). However, we do not have data to support this and further studies are necessary. Reproduction is, of course, essential for the survival of a species; when environmental, social, and physiological conditions are favourable, hormones

coordinate the integration of physiological and behavioural changes associated with reproduction (Alcock 1998). Short-term activation of adrenocortical hormone secretion associated with a low degree of physiological stress seems to be beneficial for reproduction by maintaining an animal's level of responsiveness to socio-sexual stimuli necessary for sexual arousal and reproductive activation (Carlstead and Shepherdson 1994). However, stressors suppress pulsatile secretion of luteinizing hormone (LH) and reduce the level of attractivity, proceptivity and receptivity (Pierce et al. 2008). Thus, some studies conducted in felids have shown that chronic stress can cause suppression of ovulation (Carlstead and Shepherdson 1994, Jurke et al. 1997, Mellen 2002).

To maintain a species in good condition, physiological responses to stressors (i.e., tourism pressure) should not occur over long periods of time because this has disastrous consequences for fauna. It is well known that chronically elevated glucocorticoid levels negatively impact an animal's physiology, immune system and behaviour (Sapolsky et al. 2000). However, we do not know whether the faecal cortisol metabolite levels observed in this study are indicative of decreased fitness of the species. To answer this question, it will be necessary to conduct studies that indicate whether reproductive success or other parameters are affected under conditions of high tourist pressure. One such study involving another species showed that juvenile hoatzins (*Opisthocomus hoazin*) living at ecotourist-exposed sites had lower body mass, higher mortality and higher corticosterone levels than individuals living in undisturbed areas (Müllner et al. 2004).

The wildcat is classed as 'threatened' at the national level in many European range states (IUCN 2011). Presently, ecotourism are growing, especially in protected natural areas. Therefore, it is important to evaluate in other future studies the effects of ecotourism in different wildcat populations to minimize damage to conservation of the species. This idea is further supported by the fact that previous studies have indicated that felines select zones with low human disturbance to develop their activities (Klar et al. 2007, Monterroso et al. 2009).

Based on our results, we conclude that tourism intensity increase the stress levels of wildcats. We therefore recommend the following habitat management strategies to reduce the effects of recreational activities on wildcat populations. First, taking into account the wildcat territorial size, zones where the development of recreational activities is not allowed should be maintained and supported within the protected natural areas. Second, because protected areas

are generally attractive for tourist use, it is important to minimize the effect of this use, especially during the animals' most sensitive periods. Control of visitors mainly during the period of gestation (spring) must be increased in zones where access is allowed because this is the most sensitive period for the species and is of vital importance for its survival. It is important to emphasise that there do not appear to be other active threats to the wildcat in the study area; the habitat is well preserved and is in an area far from human populations, so that interaction with domestic cats is fairly unlikely. Thus, the conservation of this area and others like it is vital for the conservation of the specie. With endangered free-ranging species, experimental disturbances and other invasive methods should for the most part be avoided. Most felids are solitary and nocturnal species (Kitchener 1995, Sunquist and Sunquist 2002); thus, their capture to take blood samples turns out to be very costly. Collection of urine samples is also difficult in cats because they frequently void by spraying (Sunquist and Sunquist 2002) and metabolism studies in the domestic cat show that the majority of adrenal and gonadal metabolites are excreted in faeces rather than in urine (Shille et al. 1990, Brown et al. 1994, Graham and Brown 1996). On the other hand, the non-invasiveness of faecal samples to measure physiological stress allows to the scientists quantify glucocorticoid levels without disturbing the animals. However, as many inconsistencies in the glucocorticoid measures are related in part to methodological problems (Busch and Hayward 2009), a careful validation for each species is obligatory (Touma and Palme 2005).

CAPÍTULO 5.

EFFECTS OF HABITAT, PREY ABUNDANCE AND COMPETING CARNIVORE ABUNDANCE ON PHYSIOLOGICAL STRESS RESPONSES IN WILDCATS

Abstract

Conservation physiology is an important tool used to understand how variations in the natural environment can invoke a physiological stress response in free-living animals. The aim of this study was to analyze how faecal cortisol metabolite levels vary in response to habitat type, prey abundance, and interspecific competition in a wild population of wildcats (*Felis silvestris*) in Northwest Spain. We collected 110 fresh scat samples from 25 wildcats in transects surveyed on foot along forest roads. Cortisol metabolites in the scat samples were analysed using an enzyme immunoassay. To determine habitat characteristics and competing carnivore abundance, we defined 110 circular plots of with 1 km of diameter with the fresh wildcat scat samples at the centre. For each plot we analysed habitat variables by using a geographic information system database, abundance of carnivore competitors (European pine marten [*Martes martes*] and red fox [*Vulpes vulpes*]) and prey abundance (wood mouse [*Apodemus sylvaticus*]). To estimate wood mouse abundance, seasonal live trappings were performed in different habitat types (deciduous forests, mature pine forests and scrublands). The results of the generalized linear model indicated that faecal cortisol metabolite levels were higher in plots where pine marten abundance was higher. In addition, wildcats showed an increase in cortisol metabolite concentrations in the plots with higher surface of pastureland, pine forests and scrubland. However, cortisol metabolite levels were lower in plots with higher surface of forest roads, deciduous forests and water. The abundance of red foxes and wood mice had lower relevance in the occurrence models. Our results indicate that faecal cortisol metabolite levels in wildcats increase in habitats with greater interference competition.

Introduction

Abiotic and biotic changes are common in the environment, and animals respond to these changes through temporal variation in their vital rates (Morris and Doak 2002) and alteration in their physiological responses (Wingfield et al. 1997). Adolph (1956) demonstrated the relationship between physiological regulation and the ability of an animal to adapt to new environmental conditions. Therefore, physiological tools serve as useful

diagnostics in conservation physiology (Wingfield et al. 1997, Wikelski and Cooke 2006, Busch and Hayward 2010).

Glucocorticoid (GC) levels are used as a hormonal measure of physiological stress (Wingfield and Romero 2001, Wikeliski and Cooke 2006). When an animal is subjected to a stressor, the hypothalamus releases adrenocorticotropic hormone (ACTH), which signals the adrenal cortex to release steroid hormones such as GCs to help overcome stressful situations (Sapolsky et al. 2000) and restore homeostasis (Möstl and Palme 2002). However, prolonged exposure to stressors causes chronic increase in GC levels and leads to detrimental 'chronic stress' (Romero 2004). Chronic stress causes depressed immune responses, reduced reproductive success, suppressed growth, or decrease survival, and can therefore, negatively affect individual fitness (Romero 2004).

Faecal glucocorticoid metabolite quantification is a non-invasive tool that provides important information about endocrine status (Palme 2005). This non-invasive method is particularly useful because samples can be obtained without disturbing the animals (Wasser et al. 2000). Thus, faecal glucocorticoid analysis is a valuable, non-invasive tool for studies on potential stressors that affect animals in natural conditions.

Habitat is an environmental factor that may affect GC levels. It is well known that habitat quality can influence the physiology and individual fitness of vertebrates (Huey 1991). Therefore, habitat change or loss can be considered as a stressor in free-living vertebrates, (Wingfield et al. 1998) and in the context of landscape ecology, physiological responses can be used to guide conservation and habitat restoration efforts (Wileski and Cooke 2006). However, few studies have explored the links between physiological responses and habitat spatial patterns (Ellis et al. 2012). In mammals, most studies have examined the effect of habitat quality on the levels of GC (e.g., kit fox, *Vulpes macrotis* and coyote, *Canis latrans*: Nelson 2005; Iberian wolf, *Canis lupus*: Barja et al. 2007; black howler monkey, *Alouatta pigra*: Martínez-Mota et al. 2007).

Food limitation may also elicit physiological stress responses in vertebrates (Ellis et al. 2012), affecting survival and limiting growth, survivorship, and fecundity (Boutin 1990). Nutritional stress is defined as a negative physiological and/or behavioural state resulting from sub-optimal quantity or quality of food available to an animal (Trites and Donnelly 2003). Thus, an increase in GC levels may be attributable to nutritional stress from low forage quality (Taillon and Côté 2008) and/or low food abundance (Foley et al. 2001).

The effects of interspecific competition may also evoke a physiological stress response (Nelson 2005). There are two types of interspecific competition: exploitation competition and interference competition (Birch 1957). However, most studies linking interspecific competition with physiological responses are based on exploitation competition (e.g., prey-predator interactions: Creel et al. 2005, Sheriff et al. 2009); there are few studies about interference competition as a stressor (e.g., intrasexual competition: Boonstra et al. 2001, habitat segregation: Nelson 2005). The competitive exclusion principle predicts that two species with identical niches cannot coexist indefinitely (Gause 1934). However, subsequent studies revealed that the coexistence of similar carnivore species can be facilitated by different strategies, such as habitat use variation, temporal segregation, or trophic changes (Bonesi et al. 2004, Barrientos and Virgós 2006, Hass, 2009). In this sense, competition between species would be higher when species whose geographic areas overlap show similar body size and feeding habits (Donadio and Buskirk 2006).

In Atlantic climatic regions, wildcats and their competitors, such as the pine marten, select forest habitats (Barja 2005b, Klar et al. 2008, Pereboom et al. 2008). The red fox, which is also a competitor of wildcats, is considered a habitat generalist (Lucherini et al. 1995). Wildcats are nocturnal, solitary, and territorial carnivores (Sunquist and Sunquist 2002), and their habitat use is often associated with prey availability (Lozano et al. 2006). In the North Iberian Peninsula, rodents are the main prey of wildcats and pine martens (Rosellini et al. 2008, Piñeiro and Barja 2011), and they are also frequently consumed by the red fox (Jędrzejewski and Jędrzejewska 1992). The feeding strategy adopted by wildcat and pine marten is that of a facultative specialist predator, although the trophic diversity of the pine marten is higher than that of the wildcat (Rosellini et al. 2007, Piñeiro and Barja 2011). However, the red fox is considered to be a trophic generalist (Jędrzejewski and Jędrzejewska 1992).

Considering the results of the studies mentioned previously, the wildcat and pine marten compete through interference competition that involves direct negative interactions, whereas the wildcat and red fox are exploitative competitors and show indirect negative interactions. Therefore, interference competition and low prey abundance may act as stressors in wildcat populations. Thus, in the present study, we examined the physiological stress response induced by the following stressors in free-living wildcats: habitat type, prey availability, and interspecific competition with the pine marten and red fox. We predicted that

the wildcats would show higher faecal cortisol metabolite levels in habitats with lower prey abundance and increased interference competition.

Materials and Methods

Transect survey to collect faecal samples

Wildcats, pine martens, and red foxes use forest roads for travelling and frequently defecate on them as a way of visual-scent marking (Robinson and Delibes 1988, Barja et al. 2001, Barja 2005a). Thus, we surveyed 300-m transects on foot along forest roads in order to collect fresh wildcat faecal samples from which we quantified cortisol metabolite levels. Furthermore, in these transects, we recorded the number of pine marten and red fox scats to estimate their abundances. To increase the probability of locating scats from different individuals and to minimise pseudoreplication, the transects were separated by a distance of 700 m. The transect surveys were conducted seasonally between May 2005 and June 2009 in 25 Universal Transverse Mercator (UTM) cells of 1 km^2 each. The transects were uniformly distributed throughout the study area, and a total area of 311.2 km^2 was surveyed. Morphological characteristics (size and shape) were used to distinguish the scats of the studied species (wildcat, pine marten, and red fox) and others carnivores in the study area (weasels [*Mustela nivalis*], stoats [*Mustela erminea*], and Iberian wolves). For each pine marten or red fox scat detected, we registered the following data: species, date, and UTM coordinates.

The transects were surveyed between sunrise and three hours thereafter to collect fresh wildcat faecal samples because microbial action and exposure to environmental conditions can alter the levels of cortisol metabolites (Millspaugh and Washburn 2003). We considered a scat to be fresh when they had a moist layer of mucus, a strong smell, and no signs of dehydration (Liu et al. 2006). We collected 10 g of sample from the fine end of each fresh scat to standardise sampling procedures. All faecal samples were frozen at -20°C for subsequent laboratory analysis. We recorded the date and UTM coordinates for each fresh wildcat faecal sample.

Habitat plots and competitor abundance

In order to determine habitat characteristics and the abundance of competing carnivores, we defined 110 circular plots with 1 km of diameter with the fresh wildcat scat

samples at the centre (Fig. 18). For each plot we analysed the following variables: (1) pine marten and red fox abundance, (2) wood mouse abundance, (3) total surface of deciduous forests, pine forests, scrublands, and pastureland (pastures and crops), (4) total surface occupied by water, and (5) total surface occupied by forest roads.

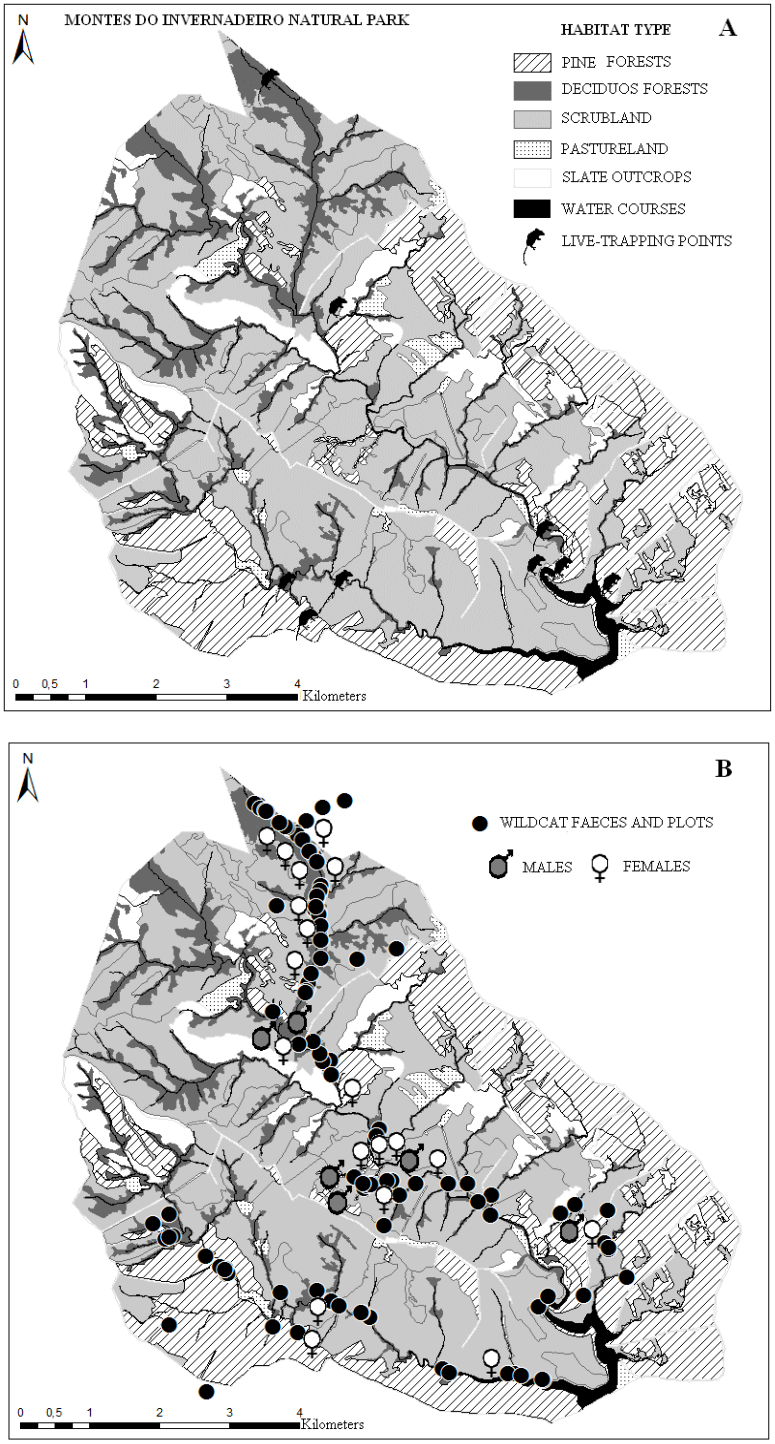
The abundance of pine martens and red foxes was obtained by counting scats of these species in each plot. To estimate wood mouse abundance in each plot, we considered its predominant habitat and the season and year in which each fresh wildcat scat was located. Thus, for each plot, we extrapolated the number of wood mice captured at the nearest trapping cell. In addition, we considered that the trapping cell had the same predominant habitat as the plot and that the captures were performed in the same season and year as the collection of the wildcat faecal sample.

The habitat variables of water surface and forest roads surface in each plot were estimated using a geographic information system (GIS) database. Location data were translated into GIS (gvSIG 1.9. Conselleria d'Infrastrucutures i Transport, Generalitat Valenciana; available at http://www.gvsig.org/web/).

Prey abundance

The wood mouse constitutes the principal prey of wildcats in the study area (Piñeiro and Barja 2011). Thus, to determine its abundance, we conducted seasonal live trappings from August 2005 to June 2007 in the three most representative habitats of the study area (deciduous forests, pine forests, and scrubland) (Fig. 18A). In each habitat, the traps were placed in three different UTM cells separated by a mean distance of 3 km. In each cell, we placed 25 Sherman traps in a grid pattern where each trap was separated by 10 m (total effort, 4,725 trap nights). The traps were left open 24 h for three consecutive days and checked every 12 h. All the traps were baited with bread soaked in oil and insulated from the heat and cold with raw wool placed raw wool that serves as insulator from the cold and the heat. The captured animals were marked with a non-toxic, waterproof, coloured spray paint to identify possible recaptures and avoid pseudoreplication. After handling, the wood mice were released at the point of capture. The research was undertaken with permission from the Xunta Galicia wildlife authority (letters of 18/04/05, 18/09/06, 13/07/07).

Fig. 18. Study area in the Northwest Iberian Peninsula. (A) Predominant habitats and trapping points for wood mice. (B) Distribution of fresh wildcat faeces and wildcat males and females in the study area.



Specific origin and individual genotyping

It is difficult to differentiate between wildcat faeces and those of feral cats or hybrids on the basis of shape and size. Therefore, it is necessary to use a multi-evidence approach including DNA methods, as reported Davison et al. (2002). In the present study, species identification through molecular analysis was conducted using a sub-sample of faeces to determine the species of origin and the reliability of the data obtained. In addition, individual genotyping was performed, according to the method used by Oliveira et al. (2009), to determine the minimum number of wildcats from which the scats originated; this information was necessary to determine whether the number of detected scats was representative of the wildcat population. To perform species identification and individual genotyping, we collected a total of 37 fresh faecal samples spread evenly throughout the study area. A total of 25 different wildcat genotypes were identified (6 males and 19 females) (Fig. 18B).

DNA extraction from the faecal samples was performed using salting-out and phenol-chloroform extraction (Sambrook et al. 1989). We assessed individual multilocus genotypes by using 12 neutral unlinked microsatellites that were isolated and characterised in the domestic cat (Oliveira et al. 2008a). Polymerase chain reaction (PCR) amplification of individual microsatellites was performed, as described by Randi et al. (2001). Allele frequencies, standard diversity indices, and observed (HO) and expected (HE) heterozygosities for each locus and population were calculated using GENETIX 4.05 (Belkhir et al. 1996-2004).

To measure the reliability of the scat identifications, we used Reliotype software (Miller et al. 2002), which was used to assess the reliability of the multilocus genotypes and to estimate the number of replicates necessary to obtain a genotype with 95% confidence. The software GIMLET (Valiere 2002) was used to estimate error rates and construct consensus genotypes from the genotyping replicates. Moreover, it was used to regroup identical genotypes from different scat samples and determine parentage between individuals (kinship).

Quantification of faecal cortisol metabolites

Cortisol metabolites were extracted using previously established methods for similar carnivore species (Brown et al. 1994, 1996, Young et al. 2004, Barja et al. 2007). The

efficiency of extraction was tested by the addition of radiolabelled hormone (3H-cortisol, 4000-8000 dpm, ICN, California, USA) to a parallel set of faecal samples, prior to extraction.

The cortisol metabolite concentrations in the faecal extracts were determined using an enzyme immunoassay. Hormone concentrations were calculated using software developed for this technique (ELISA-AID, Eurogenetic, Belgium). Standard dose-response curves were constructed by plotting the binding percentage (B/Bo × 100) against the standard hormone concentrations added.

The mean recovery percentages from faecal extracts were 95% for high concentrations and 98% for low concentrations. Parallel displacement curves were obtained by comparing serial dilutions of pooled faecal extracts with the standard curves. The results showed that both curves were parallel. Intra- and inter-assay coefficients of variation were calculated by assaying 10 replicates of a pooled faecal sample in the same assay and 10 replicates of the same sample in 10 consecutive assays. The percentages recovered for high and low concentrations were 4.5% and 7.5%, respectively. For each sample, cortisol metabolite concentrations were expressed in nanograms per gram of dry faeces.

Statistical analyses

Since we observed that the variables were not normally distributed (Shapiro-Wilk test), we log (x+1) transformed the quantitative variables prior to analyses to ensure normality and homoscedasticity. To avoid multicollinearity (Graham 2003), we performed a principal component analysis (PCA) on the basis of correlation matrices to reduce the habitat variables into a smaller number of principal components (PCA factors). The variables included in PCA were the following: deciduous forest, pine forest, scrubland and pastureland, water, and forest roads surface.

We used a generalized linear model (GLM) to analyze the independent variables (PCA factors, pine marten abundance, red fox abundance, and wood mouse abundance) influencing faecal cortisol metabolite levels in wildcats (response variable). In order to select the best GLM models, we ranked the different alternative models based on relative differences in the second order Akaike's Information Criteria (AIC). We calculated Akaike's weight (wi), which is interpreted as the weight of evidence that a model is the best approximating model (Burnham and Anderson 2002). We used the sum of Akaike weights (Σwi) for each variable to rank variables by importance (Burnham and Anderson 2002); the variables with the highest

weight ($\Sigma\omega_m = 0.95$) being more important than the other variables. Selection of candidate models followed the rule where $\Delta i \leq 2$ has substantial empirical support (Burnham and Anderson 2002).

A *P* value <0.05 was considered statistically significant. All analyses were performed with STATISTICA v.8.0 software for Windows (StatSoft Inc, Tulsa, USA).

Results

Scats from pine martens and red foxes were detected in 86 of 110 plots containing wildcat scats; the scat number per plot ranged from one to seven for both species. We captured a total of 232 wood mice during the study, and their abundance varied between 1 and 25 individuals per plot (mean 8.9 ± SD 5.8).

The PCA analysis by using habitat variables produced two orthogonal factors, which explained 55.1% of the total variance. The first factor was positively associated with the surface of pastureland and pine forests and negatively associated with the surface of forest roads and deciduous forests. The second factor was positively associated with the surface of scrubland but negatively associated with the surface occupied by water (Table 9).

Table 9. Results of the principal component analysis performed with variables used to describe the wildcat habitat. Asterisks (*) indicate the significant correlations (*P* <0.01) between the variables and factors.

Variables	Factor 1	Factor 2
Water surface	0.18	–0.80*
Forest road surface	–0.66*	0.21*
Scrubland surface	–0.04	0.66*
Deciduous forest surface	–0.67*	0.01
Pastureland surface	0.59*	0.41*
Pine forest surface	0.77*	–0.33*
Eigenvalue	2.07	1.24
% Explained variance	34.48	20.60

The levels of faecal cortisol metabolites were analyzed in 110 fresh faecal samples of wildcats. A total of 31 occurrence models were possible with the variables considered, but only 12 models were regarded as plausible (ΔAIC < 2) (Table 10).

Table 10. Relative contribution of each variable in the best models that explain the faecal cortisol metabolite concentrations (response variable) in wildcats. Number of parameters used (k), Akaike information criterion (AIC), difference between each selected model and the best model (ΔAIC), log-likelihood ratio and their respective *P*-values.

Models	k	AIC	ΔAIC	Log-likehood ratio	*P*
Factor 1	1	186.83	0	0.20	0.65
Pine marten abundance	1	186.90	0.06	0.14	0.71
Factor 2	1	186.92	0.09	0.11	0.73
Red fox abundance	1	186.96	0.12	0.08	0.78
Wood mouse abundance	1	187.04	0.20	0.00	0.95
Pine marten abundance + Factor2	2	188.05	1.22	0.99	0.61
Factor 1 + Factor 2	2	188.72	1.88	0.32	0.85
Red fox abundance + Factor 1	2	188.76	1.92	0.28	0.87
Pine marten abundance + Factor 1	2	188.77	1.93	0.27	0.87
Pine marten abundance + RFA	2	188.82	1.98	0.22	0.89
Wood mouse abundance + Factor 1	2	188.82	1.99	0.22	0.90
Red fox abundance + Factor 2	2	188.85	2.01	0.19	0.91

Factor 1, pine marten abundance, and factor 2 were the variables with the most weight in explaining faecal cortisol metabolite concentrations (Table 11). Thus, cortisol metabolite levels in the wildcat faeces were higher in plots where the pine marten abundance was higher. In addition, wildcats showed an increase in faecal cortisol metabolite concentrations in the

plots with higher surface of pastureland, pine forests, and scrubland. However, the faecal cortisol metabolite levels were lower in the plots with higher surface of forest roads, deciduous forests, and water. The abundance of red foxes and wood mice had lower relevance in occurrence models (wi < 0.3) (Table 11).

Table 11. Results of the generalized linear model with the independent variables (principal component analysis factors, wood mouse abundance, pine marten abundance, and red fox abundance) explaining the variation in faecal cortisol metabolite levels, according to the sum of the Akaike weight values. The table shows the weight across of the most parsimonious models (w_i), β coefficient, and standard error (SE) for each variable.

	Wi	β	SE	95% IC	Upper 90%	Lower 90%
Intercept		478.3	899.5	1763.0	2241.3	–1284.7
Factor 1	0.33	0.06	0.19	0.36	0.43	–0.30
Pine marten abundance	0.30	0.09	0.29	0.57	0.66	–0.48
Factor 2	0.30	0.09	0.29	0.57	0.65	–0.48
Red fox abundance	0.27	0.02	0.14	0.28	0.30	–0.26
Wood mouse abundance	0.17	0.00	0.01	0.01	0.01	–0.01

Discussion

The results of this study indicate that some habitat variables and the abundance of a carnivore competitor, such as the pine marten, were the factors that best explained the increase in faecal cortisol metabolite levels in wildcats. A habitat variable that increased physiological stress levels is the pine forest; this habitat type is an important stressor for wildcats. This result is similar to that of a study performed by Corbett (1979) who indicated that mature coniferous forests were rejected by wildcats. The increase in cortisol metabolite levels in wildcats in this habitat type may occur because it is one of the preferred habitats of the pine marten (Barja 2005b). This suggested that these species could compete for the use of space and trophic resources (rodents), which are more scarce in pine forests than in the other habitat types in the

study area (Barja and Piñeiro, unpublished data). In addition, the pine forests have greater snow depth (Dötterer and Bernhart 1996) and this hinders the wildcats' ability to hunt for rodents (Corbett 1979). Thus, wildcats avoid areas with snow (Liberek 1999).

In scrubland, wildcats showed high faecal cortisol metabolite levels. The importance of scrubland in providing wildcats with cover for shelter and richness in prey has been shown in several studies (Lozano et al. 2003, Thiel 2005, Monterroso et al. 2009). In the study area, the scrubland is the habitat that harbours a greater abundance of the wildcats' main prey, small mammals (Piñeiro and Barja, unpublished data). Therefore, in the scrubland, competition for the main trophic resource could it be high at the intraspecific level but also at the interspecific level because pine martens also seem to hunt frequently in this habitat type (Clevenger 1994).

Wildcats also showed higher faecal cortisol metabolite levels in open areas (pastureland). The frequent use of pasture and crop areas by wildcats has been indicated in different studies (Easterbee et al. 1991, Lozaro et al. 2003); these areas are mainly used during the night for foraging and hunting (Thiel 2005). The increase in faecal cortisol metabolite levels associated with this habitat may be because these areas are exposed to humans (Monterroso et al. 2009). This result concurs with those of other studies conducted with felids in captivity (Montanha et al. 2009) and with that of a study conducted with pine martens in the same study area (Barja et al. 2007), in which human presence was considered as a stressor.

In contrast, the wildcat faeces collected in zones of the study area where deciduous forests and water courses are the predominant habitats showed low cortisol metabolites concentrations; this indicates the importance of this habitat type for the species. In the study area, the deciduous forests are located in valleys crossed by rivers and permanent streams. The preference of the wildcat for riparian areas is widely known (Daniels et al. 2001, Klar et al. 2008), even in the study area (Barja and Bárcena 2005). The deciduous forests provide cover for shelter (Klar et al. 2008), e.g., tree cavities, which can be used as dens (Stahl and Artois 1991). In addition, many studies have shown the importance of watercourses in habitat selection for wildcats (Lozano et al. 2003, Barja and Bárcena 2005, Klar et al. 2008, Jerosch et al. 2010) because of the higher abundance of small mammals (hUallacháin and Madden 2011).

The cortisol metabolite levels in the wildcats were also higher when the surface of forest roads was lower. Wildcats use forest roads for travelling and leaving their territorial signals (Corbett 1979). Thus, the forest roads may act as travel corridors, especially during

times of heavy snowfall (Woods and Munro 1996), and as hunting areas, where competition for resources with other carnivores is greater because the roads are a suitable habitat for wood mice (Bellamy et al. 2000). However, the activity pattern of the wildcat in forest roads overlaps with the activity patterns of the pine marten and red fox (Corbett 1979, Clevenger 1993, Doncaster and Macdonald 1997). Therefore, when there are few forest roads, the probability of encounters with competitors such as pine martens and red foxes is greater.

In addition to habitat variables, our results show that the abundance of carnivore competitors, such as the pine marten, can act as an acute stressor in wildcats. The pine marten is a carnivore with slightly lower body size than the wildcat, but with similar prey and habitat preferences (Barja 2005b, Rosellini et al. 2008). Therefore, the increase in faecal cortisol metabolite levels in wildcats may be related to their defence of trophic and habitat resources against a competitor. Donadio and Buskirk (2006) argued that similarity in body size has been proposed to lead competitors to seek similar prey, which increases the likelihood of interference encounters. Furthermore, in the study area, both carnivores (wildcat and pine marten) prey more on the wood mouse in seasons when the rodent is most vulnerable, but not a too abundant trophic resource (Rosellini et al. 2008, Piñeiro and Barja 2011).

In the present study, the abundance of red foxes does not seem to influence cortisol metabolite levels in wildcats. This may be attributable to the generalistic character of the red fox, both at a trophic level (Jedrzejewski and Jędrzejewska 1992) and in habitat selection (Lucherini et al. 1995); this leads to lower competition between the two species. The abundance of wood mice also did not greatly influence cortisol metabolite levels. These results are consistent with those of a previous study conducted in the same area, in which it was observed that the consumption of wood mice depended on their vulnerability but did not depend on their environmental availability (Piñeiro and Barja 2011). Therefore, for wildcats, the main prey availability appears to have a lower importance than its vulnerability, as supported by the results obtained by other authors (Sladek 1973, Liberek 1999).

CONCLUSIONES GENERALES

1. El gato montés incluyó en su dieta micromamíferos, reptiles, insectos y aves. Sin embargo, los roedores constituyeron la presa principal a lo largo de todo el año, siendo el ratón de campo el roedor más depredado. El consumo de las presas mencionadas mostró una variación estacional, siendo mayor la amplitud de nicho trófico en las estaciones cálidas (primavera y verano) que en las estaciones frías (otoño e invierno).

2. El consumo del ratón de campo, su presa principal, siguió también un patrón estacional, siendo mayor en otoño e invierno. Sin embargo, la abundancia de esta especie presa no varió sustancialmente entre estaciones, aunque fue ligeramente mayor en verano y otoño. El gato montés la seleccionó más de lo esperado por su disponibilidad en el medio en otoño, invierno y primavera. Por el contrario, en verano el ratón de campo fue consumido menos de lo esperado por su disponibilidad en el medio. Estos resultados indican que la disponibilidad de la presa principal no explica el aumento en su consumo en determinadas estaciones.

3. Por otro lado, los ratones de campo tardaron más en huir lentamente en otoño e invierno, cuando su consumo fue mayor, siendo así más fáciles de capturar por el gato montés. De este modo, el patrón estacional de consumo de la presa principal se relaciona con la vulnerabilidad de la misma.

4. La diversidad de dieta a lo largo del año (principalmente durante las estaciones cálidas), la variación estacional en el consumo de su presa principal, así como, el aumento estacional en el consumo de su presa principal en relación con la facilidad de detección, captura y manipulación, son los factores que indican que el gato montés en el área de estudio no es un especialista trófico. El felino se puede definir como un especialista facultativo, al sincronizar el consumo de su presa principal con la estación en la que ésta se encuentra más débil y vulnerable; y por tanto, es más fácil de capturar.

5. El gato montés seleccionó los sustratos vegetales como postes de marcaje para depositar sus marcas fecales. Sin embargo, no todos los sustratos resultaron ser igual de óptimos para el marcaje fecal de la especie. Entre todas las plantas, el gato seleccionó aquellas que presentaban mayor diámetro, eran más conspicuas visualmente y pertenecían al grupo de las herbáceas, defecando sobre algunas especies vegetales más de lo esperado por su disponibilidad en el medio (*B. sylvaticum*, *E. cinerea*, *P. tridentatum*, *Genista sp.*, *Rubus sp*. y *H. lasianthum*). Las gramíneas como *A. elatius*, *B. media* y *H. lanatus* fueron marcadas con heces menos de lo esperado por azar. La altura de las plantas no fue determinante en su selección como poste de marcaje. El gato montés no distribuye aleatoriamente sus marcas fecales en el medio, sino que selecciona en su territorio aquellos sustratos vegetales cuyas características físicas maximizan su detectabilidad por posibles intrusos y por parejas potenciales.

6. El número de excrementos depositados por el gato montés con función de marcas oloroso-visuales (sobre sustratos conspicuos, sobre sustratos elevados, en cruces de caminos y/o en letrinas) fue mayor en aquellas zonas del área de estudio donde la abundancia de roedores, su presa principal, fue mayor. Estos resultados indican que una de las funciones de las marcas fecales en esta especie es la defensa de áreas favorables de caza. Al depositar las marcas fecales en sustratos y zonas que maximizan su detectabilidad por otros congéneres, el felino está protegiendo su principal recurso trófico y posiblemente reduciendo la competencia intraespecífica.

7. Las perturbaciones humanas, la reproducción de las hembras y la dispersión de los individuos jóvenes constituyen agentes estresantes para el gato montés en el área de estudio. Así, los niveles de metabolitos del cortisol fecal fueron más elevados en aquellas zonas del parque natural donde la presión turística era más intensa, la zona de uso público restringido. Por el contrario, los niveles de metabolitos del cortisol fecal fueron bajos en la zona de reserva integral, donde apenas existían perturbaciones humanas debidas al turismo. Los niveles de metabolitos del cortisol fecal aumentaron también en otoño cuando ocurría la dispersión de los individuos jóvenes y en primavera (gestación de las hembras) cuando las concentraciones de progesterona fecal eran más elevadas.

8. Aunque los espacios naturales protegidos son un atractivo para el turismo, es importante minimizar los efectos del mismo sobre la fauna silvestre, especialmente durante los periodos más sensibles para los animales. Así, se recomienda que en las zonas del parque natural donde se permite el acceso a los visitantes, su número debe ser especialmente controlado durante el periodo de gestación, por ser ésta una etapa crucial para la supervivencia de la especie. Además, la zona de reserva integral debe mantenerse libre de perturbaciones humanas.

9. Los niveles de metabolitos del cortisol fecal fueron mayores en aquellas zonas del territorio del gato montés donde los pastos, los pinares y el matorral ocupaban una mayor superficie, así como en aquellas zonas donde la abundancia de una especie competidora por interferencia como la marta europea era mayor. Sin embargo, en las zonas donde los bosques caducifolios, los caminos forestales y el agua ocupaban una gran superficie las concentraciones de metabolitos del cortisol fecal fueron menores. La abundancia de zorro, una especie generalista en lo que se refiere a requerimientos de hábitat y alimento, y la abundancia de su presa principal, los roedores, fueron factores que tuvieron menor peso en explicar el aumento en los niveles de estrés fisiológico en el gato montés.

10. El gato montés, como la mayoría de los felinos, es una especie solitaria, nocturna y difícil de observar y capturar en el campo, lo que sin duda dificulta su estudio en condiciones naturales. Por tanto, el uso de una metodología no invasiva y la validación de técnicas que permitan cuantificar los niveles de glucocorticoides en heces en relación con factores ecológicos y comportamentales, supone un gran avance a la hora de conocer cuál es la condición física de los individuos sin necesidad de capturar a los animales. Estos estudios son de vital importancia para conocer las respuestas fisiológicas del felino a diferentes estresores que hay en el medio donde vive (perturbaciones humanas, competencia con otras especies, escasez de alimento) y cuáles son los potenciales peligro para su conservación. La cuantificación de los niveles de hormonas esteroides en muestras fecales es un método no invasivo que permite evaluar la respuesta de estrés fisiológico y el estado reproductor en una especie esquiva como es el gato montés.

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ANEXOS

ANEXO I

Los capítulos de esta tesis doctoral se encuentran publicados o bajo revisión en las siguientes revistas incluidas en SCI (*Science Citation Index*):

- Capítulo 1.- **Piñeiro A.**, Barja I., 2011. Trophic strategy of the wildcat *Felis silvestris* in relation to seasonal variation in the availability and vulnerability to capture of *Apodemus* mice. *Mammalian Biology*, 76: 302-307.

- Capítulo 2.- **Piñeiro A.**, Barja I. Plant physical features selected by wildcats as signal posts: An economic approach to fecal-marking. *Naturwissenschaften*. In re-review.

- Capítulo 3.- **Piñeiro A.**, Barja I. Evaluating the function of wildcat faecal marks in relation to the defence of favorable hunting areas. *Acta Ethologica*. Under review.

- Capítulo 4.- **Piñeiro A.**, Barja I., Silván G., Illera J.C. Effects of tourist pressure on reproduction and physiological stress response in wildcats: management implications for species conservation. *Wildlife Research*. In re-review.

- Capítulo 5.- **Piñeiro A.**, Barja I., Otero, G.P., Silván G., Illera J.C.. Effects of habitat, prey abundance and competing carnivore abundance on physiological stress responses in wildcats. *Acta Oecologica*. Under review.

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Original Investigation

Trophic strategy of the wildcat *Felis silvestris* in relation to seasonal variation in the availability and vulnerability to capture of *Apodemus* mice

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ABSTRACT

The aim of this study was to assess the trophic strategy of the wildcat (*Felis silvestris*) by examining the availability and vulnerability of its main prey. Live traps were used to estimate *Apodemus* mouse availability. The vulnerability to capture of wildcat main prey – *Apodemus* mice – was studied by focal sampling of live-trapped individuals; slow escape behaviour and body weight were used as indicators of vulnerability to capture. The seasonal consumption of *Apodemus* mice did not depend on their availability, although seasonality was the only factor that explained the variation in slow escape behaviour, which was more commonly seen in the autumn when the consumption of these mice was higher. Variation in mouse body weight was related to reproductive condition but not to seasonality. These results indicate that the wildcat is a facultative specialist in the consumption of *Apodemus* mice, with vulnerability to capture the main factor determining the rate of mouse consumption.

Introduction

The composition of the diet of the wildcat (*Felis silvestris*) is one of the best known features of the species' ecology throughout its range (Stahl and Leger, 1992; Sunquist and Sunquist, 2002; Lozano et al., 2006). Rabbits are the main dietary component in some regions. For example, in eastern Scotland, wildcats feed almost exclusively on rabbits (Corbett, 1979), and in the south and centre of Spain rabbits are consumed with greater frequency than rodents (Gil-Sánchez et al., 1999; Malo et al., 2004). Nevertheless, most of the studies conducted in Europe have reported that rodents, especially murines and microtines, are the main dietary items (France: Condé et al., 1972; Caucasus: Nasilov, 1972; Italy: Ragni, 1978; Carpathians: Kožená, 1990; Portugal: Sarmento, 1996; Carvalho and Gomes, 2004; Spain: Aymerich, 1982; Moleón and Gil-Sánchez, 2003; Urra, 2003). The inclusion of Murinae and Microtinae in the diet also varies with latitude, with higher consumption of the former in southerly locations and higher consumption of the latter in more northerly areas (Lozano et al., 2006).

Most studies suggest that the wildcat is a trophic specialist in the consumption of rodents. A species is considered a trophic specialist when it feeds almost entirely on one prey species or group of prey animals such as "rodents", and when it shows this preference regardless of the prey's availability or vulnerability to capture (Holling, 1959; Glasser, 1982). Such predators are said to show a type II (hyperbolic) functional response (Holling, 1959). A trophic opportunist, however, consumes the food most at hand in each season and area, changing its diet depending on food availability and vulnerability to capture. When the abundance of one prey type diminishes, opportunist predators begin to take a more abundant species; they therefore show a type III functional response (S-shaped) (Holling, 1959; Glasser, 1982; Angelstam et al., 1984). A facultative specialist, in contrast, may behave more opportunistically, i.e., by changing a key food item when more profitable prey is available (Glasser, 1982).

Prey availability and vulnerability are not key factors in the dietary intake of specialist species; thus, when these factors vary between seasons, no differences should be seen in the wildcat diet. However, trophic ecology studies regarding the quantitative availability of wildcat preys are scarce and no studies have been performed on the vulnerability to capture of its main prey. Although these aspects are essential for defining the trophic strategy of the species, only Malo et al. (2004) have evaluated the trophic strategy of the wildcat in any depth. Nonetheless, in this study the availability and vulnerability of the main prey species (rabbits, and small mammals in areas where rabbits were not available) were not analysed. The present study was designed to test the hypothesis that wildcats are specialist predators of the *Apodemus* mouse. If they are: (1) although other profitable prey species are present in the environment, the *Apodemus* mouse should remain the main prey item year round, (2) *Apodemus* mouse consumption should not vary

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among seasons according to the environmental availability of the species or its vulnerability to capture.

Material and methods

Study area

The study area was the Montes do Invernadeiro Natural Park, which covers an area of 5722 ha. This mountainous area is located in the northwest of the Spain, where it forms part of the transition zone between the Eurosiberian and Mediterranean biogeographical regions. The latter is manifested by the alternation between Mediterranean plant communities and Atlantic relict forests (Pulgar, 2004). Scrubland dominates the plant community, which mainly consists of heather (*Erica australis*), prickled broom (*Pterospartum tridentatum*) and sandling (*Halimium lasianthum*). Large areas are occupied by repopulated forests of Scot pine (*Pinus sylvestris*), whereas the original forest – mainly associations of oak (*Quercus robur*), birch (*Betula celtiberica*) and holly (*Ilex aquifolium*) – persists in valleys and along watercourses. The Montes do Invernadeiro Natural Park is located in a region of low human density. Within the park itself there are only a few isolated and uninhabited houses.

Scat collection

Scats were collected seasonally from August, 2005 to June, 2007 by establishing transects along roads in optimal areas for the species; these areas were identified in previous studies on wildcat distribution and habitat selection in the study area (Barja and Bárcena, 2005). In each season we surveyed on foot transects 300 m long in 22 UTM cells of 1 km^2 each (total distance surveyed = 98.4 km in 328 transects). We surveyed in spring 152 trasects, in summer 38, in autumn 72 and in winter 66. These differences between seasons were due to adverse weather conditions at certain months of year. Wildcat mean home range size is of 400 ha (Genovesi and Boitani, 1992; Scott et al., 1992) and there is intersexual territory overlap (Stahl et al., 1988). Therefore, in order to increase the likelihood of obtaining faecal samples from different individuals and to minimise pseudoreplication, the itineraries were established in four zones of the study area set far apart from one another and in each zone the transects of 300 m were separated by a distance of 1 km. Furthermore, it is important to highlight that faecal sampling on transects avoid the error caused by overrepresentation of particular individuals because increases the probability to obtain samples from different individuals (Huber et al., 2003).

Wildcat scats were differentiated from those of other sympatric carnivores (European pine marten, red fox and badger) by their size and shape. The scats of the wildcat, domestic cat and their hybrids are very similar and their differentiation is complex. However, the human population nearest to the study area is 7 km to the south; the presence of domestic cats would, therefore, at the very least be greatly reduced. During this study, 24 cats were observed and another eight photographed using camera traps; all showed typical wildcat external morphology (Stahl and Leger, 1992; Kitchener, 1995; Sunquist and Sunquist, 2002). Wildcats are reported to show deposit their faeces as marks in conspicuous locations and not bury them (Corbett, 1979; Barja and Bárcena, 2005), while domestic cats tend to bury their faeces in sympatric zones (Corbett, 1979; Schauenberg, 1981). None of the faeces detected along the transects were buried; indeed, they often had a marking function, being deposited in prominent locations. This difference in behaviour therefore differentiates their faeces. In addition, recent studies conducted in different European populations on interbreeding between wildcats and feral domestic cats indicate that hybridisation is a problem not very frequent (Randi et al., 2001; Pierpaoli et al., 2003; Lecis et al., 2006; Oliveira et al., 2008). The presence of domestic cats and hybrids in the study area was therefore not very likely, providing a guarantee of the origin of the scats studied.

Diet analysis

The scats were cleaned in the laboratory following conventional procedures (Reynolds and Aebischer, 1991). After drying, the different macroscopic components were separated, weighed and identified. The consumed prey animals were classified into five categories depending on the indigestible remains: small mammals (rodents and insectivores), other mammals (rabbits and hares), reptiles, birds and insects.

The mammal species were identified from their skulls, jaws and/or teeth using keys (Dueñas and Peris, 1985; Blanco, 1998). When no bone remains were present, the cuticle patterns of the hairs were compared to those in reference manuals (Faliu et al., 1980; Teerink, 1991) and with reference hairs collected in the study area.

The composition of the diet was expressed in terms of frequency of occurrence and percentage of consumed biomass. To estimate the consumed biomass of the five main categories, the following Lockie's correction factors were used (Lockie, 1959; Stahl and Leger, 1992): small mammals (13.8), reptiles (45), birds (35), insects (5), rabbits (43) and hares (50). The consumed biomass of each prey species was estimated by multiplying its frequency of occurrence by its mean weight (Blanco, 1998). At the family and genus levels, consumed biomass was estimated using the mean weight of the corresponding member species.

Availability and vulnerability to capture of Apodemus mice

To estimate the availability of *Apodemus* mice, a live-trapping campaign were performed each season (between August, 2005 and June, 2007) in the three most representative habitats (deciduous forest, mature pine woods and brushwood). In each habitat, three trapping sites were selected far from one another. A grid containing 25 Sherman traps, separated by 10 m and covering an area of 0.25 ha was defined at each trapping site. In each habitat the traps were left out for three consecutive nights and checked every 12 h. The total effort therefore involved 4725 trap-nights. Bread impregnated with oil was used as bait.

For each captured *Apodemus* mouse the following data were registered: species, sex, body weight, relative age (individuals weighing less than 15 g were considered juveniles or subadults and those weighing more than 15 g as adults), breeding condition (breeding or non-breeding individuals) and habitat type (deciduous forest, mature pine wood and brushwood). The mice were reliably sexed and their breeding status determined on the basis of several indicators (Gurnell and Flowerdew, 1994). All the captured individuals were marked with inoffensive paint to differentiate them in later recapturing.

Slow escape behaviour (reflecting the vulnerability to capture by wildcats) of 104 *Apodemus* mice (71 adults, 18 subadults, 7 young, 8 of undetermined age) was recorded by focal sampling (one-zero recording) (Martin and Bateson, 1986). Every focal animal was observed at 5 s intervals over 2 min. These live-trapped individuals were not freed directly from the traps, first were handled (weighed, sexed, marked and observed their breeding condition), to avoid that hypothermia during cold seasons affected the results. The handling time of each animal was approximately 5 min. After the handling each individual was released at the place of capture, but in an area free of vegetation (to aid visibility), and the slow escape behaviour recorded. Although the number of recaptures was low (12.5%) dur-

Table 1
Diet composition of wildcats in the northwestern Iberian Peninsula.

		Genre/Specie	FO[a]	CB%[b]
Mammals	Rodents	Murinae	26	3.2
		Apodemus sp. (*A. sylvaticus* and *A. flavicollis*)	114	24.0
		Mus sp.	73	5.1
		Arvicola sp.	5	3.8
		Water vole (*Arvicola terrestris*)	2	0.8
		Microtus sp.	27	4.2
		Field vole (*Microtus agrestis*)	1	0.2
		Cabrera vole (*Microtus cabrerae*)	2	0.6
		Lusitanian pine vole (*Microtus lusitanicus*)	4	0.4
		Bank vole (*Clethrionomys glareolus*)	4	0.5
		Snow voles (*Chionomys nivalis*)	6	2.3
		Fat dormouse (*Glis glis*)	29	2.1
		Garden dormouse (*Eliomys quercinus*)	13	3.2
		Red squirrel (*Sciurus vulgaris*)	26	38.0
		Total items	332	88.4
	Insectivores	*Crocidura* sp.	8	0.3
		Greater white-toothed shrew (*Crocidura russula*)	1	0.0
		Sorex sp.	10	0.5
		Pygmy white-toothed shrew (*Suncus etruscus*)	2	0.0
		Water shrew (*Neomys fodiens*)	7	0.5
		Iberian mole (*Talpa occidentalis*)	1	0.3
		Total items	29	1.6
	Other mammals	Rabbit (*Oryctolagus cuniculus*)	7	1.6
		Hare (*Lepus granatensis*)	1	0.3
Birds			8	1.5
Reptiles			25	6.1
Insects			20	0.5
N° scats analysed			200	

[a] Frequency of prey occurrence in scats.
[b] Percentage of consumed biomass.

ing the experiment, we did not consider them for statistical analysis in order to avoid bias related with the fact that recaptured animals are more stressed than those captured for the first time and also to avoid problems of pseudoreplication.

Data analysis

To determine the relationship between the consumption of *Apodemus* mice by wildcats and their seasonal abundance, the number of scats containing *Apodemus* remains every season (ObsF) was recorded. Since the scat number collected in each season was different, the figures were corrected using the equation:

$$\mathrm{ObsF}* = \mathrm{ObsF} \times I_c; \quad I_c = \frac{\bar{N}}{N} \tag{1}$$

where ObsF* is the corrected number of scats with *Apodemus* remains, I_c is the correction index, N is the total number of scats analysed per season, and $\bar{N}$ the annual mean number of scats analysed.

The expected percentages of scats with *Apodemus* remains (ExpF%) in each season were calculated according to the following formula:

$$\mathrm{ExpF\%} = \frac{A_s \times 100}{A_t} \tag{2}$$

where A_s is the seasonal number of captured *Apodemus* and A_t the total number of captured *Apodemus* in the study area.

The expected frequencies (ExpF) of scats with *Apodemus* remains per season were calculated with the following formula:

$$\mathrm{ExpF} = \sum \frac{\mathrm{ObsF}^* \times \mathrm{ExpF\%}}{100} \tag{3}$$

To compare the overall dietary diversity (trophic niche breadth) in different seasons, the *B* index of Levins (1968), which varies from 1 (narrowest niche) to 5 (broadest possible niche), was calculated for the five food categories. The index was applied to percentage of occurrence of the main food categories (small mammals, reptiles, insects, birds, other mammals) in the whole diet.

Slow escape behaviour and the body weight of the individuals (dependent variables) were analysed via general linear models. In both models the following variables were used as fixed factors: season (spring: April–June, summer: July–September, autumn: October–December, winter: January–March), sex, age (adults, young or subadults), reproductive condition (breeding or non-breeding) and habitat (deciduous forest, mature pinewood or brushwood). Turkey's post hoc test was used for the pairwise comparison of seasonal means. Significance was set at $P < 0.05$. All calculations were performed using SPSS v. 15.0 software for Windows (SPSS Inc., Chicago, IL, U.S.A.).

Results

General remarks

A total of 200 wildcat scats (422 prey items) were used in the dietary analysis (spring: 27, summer: 60, autumn: 55 and winter: 58). The mean number of prey animals per scat was of 2.3. Small mammals were the most important prey item (frequency of occurrence 85.6%), followed by reptiles (5.9%), insects (4.7%), other mammals (1.9%) and birds (1.9%) (Table 1) ($\chi^2 = 1119.9$, df = 4, $P = 0.0001$, $N = 422$).

Among the mammals, rodents (frequency of occurrence 90.0%) were consumed in preference to insectivores (7.8%) and other mammals (rabbits and hares) (2.2%) ($\chi^2 = 534.5$, df = 2, $P = 0.0001$, $N = 369$). The consumption of Murinae was significantly higher than that of Microtinae (frequency of occurrence 80.7% compared to 19.3%) ($\chi^2 = 99.4$, df = 1, $P = 0.0001$, $N = 264$), with *Apodemus* being the genus most preyed upon (reaching a frequency of occurrence for all Murinae eaten of 61.0%) (Table 1) ($\chi^2 = 9.0$, df = 1, $P = 0.003$, $N = 187$).

Table 2
Seasonal variation in wildcat prey consumption (five categories) and trophic niche breadth (Levins index).

Seasons	*N*	Levins index	Small mammals		Reptiles		Insects		Birds		Other mammals	
			FO	CB%	FO	CB%	FO	CB%	FO	CB%	FO	CB%
Spring	27	2.5	51	56.2	6	25.6	3	1.2	1	2.9	4	14.1
Summer	60	2.2	97	60.0	14	30.6	12	2.8	3	4.7	1	1.9
Autumn	55	1.2	113	89.7	2	5.2	2	0.6	1	2.0	1	2.5
Winter	58	1.5	100	79.8	3	7.8	3	0.9	3	6.1	2	5.4

N: Number of scats, FO: frequency of prey occurrence in scats, CB%: percentage of consumed biomass.

Table 3
Seasonal variation in the consumption of different genus of small mammals (%) by wildcats.

Seasons	*N*	Apo	Mus	Micr	Arv	Sci	Clet	Chio	Eli	Gli	Total
Spring	37	37.9	32.4	16.2	5.4	2.7	2.7	0	0	2.7	100
Summer	81	38.3	22.2	5	2.5	13.6	1.2	1.2	8.6	1.2	100
Autumn	104	35.6	20.2	12.5	2.9	3.8	1.9	2.9	4.8	15.4	100
Winter	84	38.1	26.2	13.1	0	11.9	0	2.4	1.2	7.1	100

Apo: *Apodemus*; Mus: *Mus*; Micr: *Microtus*; Arv: *Arvicola*; Sci: *Sciurus*; Clet: *Clethrionomys*; Chio: *Chionomys*; Eli: *Eliomys* and Gli: *Glis*.

Seasonal patterns

The consumption of small mammals showed seasonal variation, with more being taken in the autumn (frequency of occurrence 95.0%) and winter (90.1%) than in the spring (78.5%) or summer (76.4%) (Table 2) ($\chi^2 = 174.0$; df = 3, $P = 0.0001$, $N = 361$). The highest consumption of reptiles occurred during summer (11.0%) and spring (9.2%), followed by winter (2.7%) and autumn (1.7%) ($\chi^2 = 14.2$, df = 3, $P = 0.003$, $N = 25$). The consumption of birds and other mammals also experienced seasonal variation, becoming significantly higher in winter (3.7%) and spring (6.2%). Although insects made up occasional prey items, these were better represented in the summer diet (frequency of occurrence 9.4%) than in other seasons (Table 2). Taking into account the main prey categories, the trophic niche breadth (*B*) was higher in the hot seasons (spring: 2.5, summer: 2.2) than in the cold seasons (autumn: 1.2, winter: 1.5).

In spring, *Apodemus* was the most consumed prey item, followed by *Mus*, *Microtus*, *Arvicola*, *Sciurus*, *Clethrionomys* and *Glis* ($\chi^2 = 35.5$, df = 6, $P = 0.0001$, $N = 37$). In autumn, *Apodemus* and *Mus* were the main prey, followed by *Glis*, *Microtus*, *Eliomys*, *Sciurus*, *Arvicola*, *Chionomys* and *Clethrionomys* ($\chi^2 = 94.9$, df = 8, $P = 0.000$, $N = 104$). In summer, *Apodemus* was more commonly consumed than *Mus* and, to a lesser extent, the wildcats preyed on *Sciurus*, *Eliomys*, *Glis*, *Microtus*, *Arvicola*, *Chionomys* and *Clethrionomys* ($\chi^2 = 87.1$, df = 8, $P = 0.000$, $N = 81$). In winter *Apodemus* was also the most common prey item, followed by *Mus*, *Microtus*, *Sciurus* (11.9%), *Glis*, *Chionomys* and *Eliomys* (Table 3) ($\chi^2 = 63.5$, df = 6, $P = 0.000$, $N = 84$).

Availability of Apodemus mice

A total of 232 mice belonging to the genus *Apodemus* were captured during the live trapping campaign. The abundance of mice was higher in summer (30.6%) and autumn (26.7%) than in winter (21.6%) or spring (21.1%) (Table 4), although these differences were not significant ($\chi^2 = 5.7$, df = 3, $P = 0.128$, $N = 232$). More adult individuals (>15 g) were captured in summer (31.2%) and spring (24.7%) than in autumn (23.7%) or winter (20.4%) (Fig. 1), but these differences were not significant either ($\chi^2 = 2.3$, df = 3, $P = 0.519$, $N = 93$). However, young individuals (<15 g) were more abundant in autumn (38.0%) and winter (24.0%) than in spring (20.0%) or summer (18.0%) (Fig. 1) ($\chi^2 = 4.9$, df = 3, $P = 0.181$, $N = 50$).

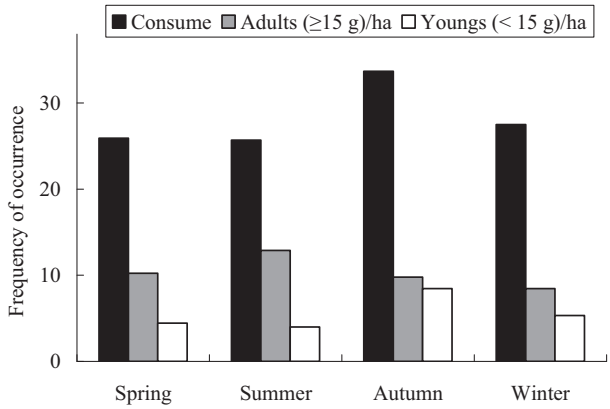

Fig. 1. Number of wildcat scats with *Apodemus* remains by seasons and compared to the abundance of adult and young *Apodemus* mice in the environment.

The number of breeding individuals of *Apodemus* was significantly higher in the hot seasons (spring and summer) (86.5%) than in the cold seasons (autumn and winter) (13.5%) ($\chi^2 = 19.7$, df = 1, $P = 0.000$, $N = 37$). The number of non-breeding individuals of *Apode-*

Table 4
Seasonal variation in the consumption of *Apodemus* mice (observed frequencies) by wildcats and their expected consumption (expected frequencies) in relation to their environmental availability (see Section Method).

	N	ObsF	I_c	ObsF*	ObsF%*	ExpF	ExpF%	*A*
Spring	27	14	1.85	25.9	23.2	23.6	21.1	49
Summer	60	31	0.83	24.7	22.1	34.2	30.6	71
Autumn	55	37	0.91	33.7	30.1	29.9	26.7	62
Winter	58	32	0.86	27.5	24.6	24.1	21.6	50
Total	200	114		111.8	100	111.8	100	232

N: total number of scats analysed per season. ObsF: number of scats with *Apodemus* remains. I_c: applied correction index. ObsF*: corrected number of scats with *Apodemus* remains. ObsF%*: corrected observed percentages of scats with *Apodemus* remains. ExpF: expected frequencies of scats with *Apodemus* remains. ExpF%: expected percentages of scats with *Apodemus* remains. *A*: abundance of *Apodemus* in the environment.

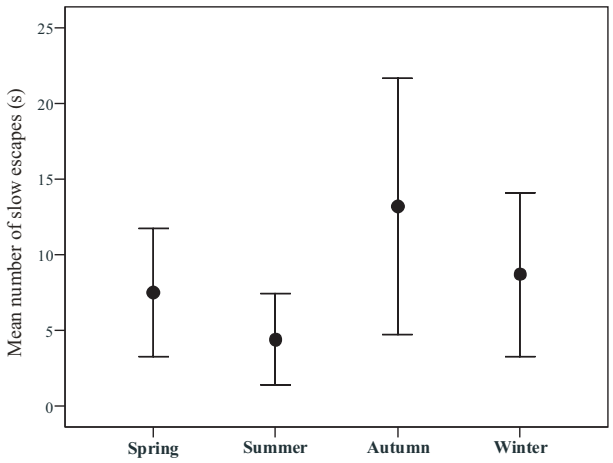

Fig. 2. Slow escape behaviour in *Apodemus* mice per season (mean ± SE).

mus was higher in the cold seasons (66.3%) than in the hot seasons (33.7%) ($\chi^2 = 10.8$, df = 1, $P = 0.001$, $N = 101$).

Consumption of Apodemus mice in relation to their availability and vulnerability to capture

The seasonal consumption of *Apodemus* mice did not depend on their availability. In the spring (23.2 vs. 21.1%), autumn (30.1 vs. 26.7%) and winter (24.6 vs. 21.6%), *Apodemus* mice were more frequently consumed than expected for their availability (Table 4). However, in summer they were consumed at a frequency lower than expected for their availability (22.1 vs. 30.6%) (Table 4, Fig. 1).

The general linear model indicated seasonality to be the only factor explaining the variation in slow escape behaviour. Slow escape behaviour was more frequently showed in autumn (13.2 ± 4.2 s), followed by winter (7.7 ± 2.3 s), spring (7.5 ± 2.1 s) and summer (4.4 ± 1.5 s) (Fig. 2) (factorial ANOVA: $F = 3.876$, df = 3, $P = 0.018$, $n = 104$). The only factor that explained the weight variation of the mice was their breeding condition (breeding: 25.8 ± 0.8 g, non-breeding: 23.9 ± 0.7 g) (factorial ANOVA: $F = 4.577$, df = 1, $P = 0.038$, $n = 104$), but weight did not vary markedly with season (spring: 24.2 ± 1.4 g, summer: 23.1 ± 0.9 g, autumn: 21.7 ± 1.3 g, and winter: 20.6 ± 1.1 g) (factorial ANOVA: $F = 0.540$, df = 3, $P = 0.657$, $n = 104$).

Discussion

This study showed that rodents, mainly *Apodemus* mice, are the wildcat's most common prey species in the study area. These results agree with those reported in previous studies from other regions of the Iberian Peninsula (Aymerich, 1982; Sarmento, 1996; Moleón and Gil-Sánchez, 2003; Carvalho and Gomes, 2004) and Europe (Condé et al., 1972; Ragni, 1978; Kožená, 1990; Liberek, 1999; Biró et al., 2005). In Mediterranean areas, where the density of rabbits is low or nil, wildcats select rodents, as occurs in many Eurosiberian regions of Europe (Kožená, 1990; Sarmento, 1996; Moleón and Gil-Sánchez, 2003; Malo et al., 2004; Biró et al., 2005). Nevertheless, when rabbits are abundant, they become the main prey species (Malo et al., 2004).

In the present study, the seasonal consumption of *Apodemus* mice did not depend on the environmental availability of this prey item. In autumn, winter and spring wildcats consumed more *Apodemus* mice than would be expected for their availability. In summer, however, this prey species was negatively selected, its consumption being lower than that expected for its availability. These results disagree with those reported by Stahl (1986) and Stahl and Leger (1992), who indicated that the consumption of rodents by the wildcat depends on the formers' temporal and spatial availability in the environment.

The present results show that there were more young *Apodemus* mice in the environment in autumn and winter, therefore, we hypothesize that their scant anti-predator experience makes their capture easy for predators. The individuals that survived in these seasons would be weaker, less agile and therefore less likely to reach their burrows before being caught by a predator.

In summer, the lower-than-expected consumption of *Apodemus* mice might be explained by the higher trophic diversity on offer to wildcats; prey items absent or scarce in the other seasons (e.g., insects), or that might be difficult to detect and capture at other times of year (e.g., hibernating reptiles) would be more abundant. The taking of these two prey items (reptiles and insects) may provide an advantage in terms of energy optimisation.

Catchability is a key parameter in prey selection (Stephens and Krebs, 1986). One anti-predator strategy of prey animals consists of simply running away from their predators (Lima and Dill, 1990), but this capacity can be diminished at certain times of year due to a shortage of food resources or adverse meteorological conditions, both of which can weaken prey animals. In the present study, the *Apodemus* mice (a basic resource for wildcats and other medium sized carnivores such as the European pine marten [e.g. see Rosellini et al., 2008]), showed slower escape behaviour during the autumn and winter. Thus, at this time of the year the probability of their capture by predators is at its highest; prey capture success is therefore greater and at lower cost to the predator (Caraco, 1980; Williams and Nichols, 1984; Belovsky et al., 1989). Wildcats seem to synchronise the consumption of their main prey with the season when it is most vulnerable. The results seem to show that the population of *Apodemus* in autumn to be made up of generally younger or thinner individuals and of others not in their top physical condition, i.e., animals less likely to be able to avoid predators.

During the cold seasons the mice could be weaker due to the high energy costs of the past reproductive period (spring and summer in the study area) and the effort used in the searching for food and refuge. In another hand, the wounds inflicted by dominant males on their subordinates during the reproductive season might also limit the survival chances of the latter (Torre et al., 2002). In autumn and winter, when the *Apodemus* slow escape time was longer, the wildcat handling time should decrease because the pursuit phase is shorter, meaning a better energy balance for the predator (Kacelnik and Bernstein, 1994).

The results therefore suggest that the wildcats of the study area follow a facultative trophic strategy with respect to the consumption of *Apodemus* mice, their vulnerability to capture being the main factor that determines the pattern of their consumption. The same conclusion has recently been reached by Malo et al. (2004) in a study conducted in the centre of the Iberian Peninsula. Nevertheless, these authors did not control for the abundance of rodents and rabbits (only for their presence or absence), nor their vulnerability to capture.

The idea of facultative specialisation proposed in this study is supported by the following results: (1) dietary diversity was greater in the hot seasons (spring and summer) than in the cold seasons (autumn and winter); in the hot seasons, the wildcats shifted their diet from *Apodemus* mice to other available prey species such as reptiles, insects and birds; (2) significant variations in small mammal consumption was seen among seasons, supporting the idea that the wildcats in the study area are not specialist species; and (3) at certain times of year the wildcats preyed more than expected on *Apodemus* mice in relation to their environmental availability, probably because their detection, capture and manipulation were easier. These results would not be expected of a trophic specialist species.

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ANEXO II

Artículos científicos publicados en los cuales la doctoranda ha colaborado como coautora y cuya temática guarda relación con la de la presente tesis doctoral:

- Barja I., Silván G., Rosellini S., **Piñeiro A**., González-Gil A., Camacho L., Illera J.C., 2007. Stress physiological responses to tourist pressure in a wild population of European pine marten. *The Journal of Steroid Biochemistry and Molecular Biology*, 104: 136-142.

- Rosellini S., Barja I., **Piñeiro A**., 2007. Distribución y hábitos alimenticios de la marta (*Martes martes*) en el Parque Natural Os Montes do Invernadeiro (Galicia, NO de España). *Galemys*, 19: 99-114.

- Rosellini S., Barja I., **Piñeiro A.**, 2008. The response of European pine marten (*Martes martes* L.) feeding to the changes of small mammal abundance. *Polish Journal of Ecology*, 56: 497-504.

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Stress physiological responses to tourist pressure in a wild population of European pine marten ☆

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Abstract

The tourist pressure in natural parks is a potential source of stress and may cause an increase in the adrenal activity of wild populations of European pine marten (*Martes martes*). Seventy-six faecal samples were collected during 15 months in a natural park of Northwest Spain. Analysis of faecal DNA was used for the specific identification using the PCR-RFLPs technique. Faecal steroid determinations were performed by EIA. Natural park was divided in three areas: free entry, restricted area, and integral reservation, and number of daily human visitors recorded. Faecal glucocorticoid metabolite levels (ng/g dry faeces) were significantly higher in spring (56.36 ± 19.62) and summer (31.27 ± 11.98) compared to autumn (15.33 ± 6.89) and winter (11.13 ± 3.30). These data are closely related to daily number of visitors (spring: 3204, summer: 1672, winter: 646, autumn: 551). Androgen, progestin and oestrogen levels were also significantly higher in spring (reproductive season) showing values of 43.62 ± 18.6, 154.31 ± 53.50 and 829.62 ± 456.1, respectively. Glucocorticoid levels were significantly lower in integral reservation (15.95 ± 3.56) compared to restricted (31.4 ± 16.30) and free entry areas (41.59 ± 12.73), respectively. Wild populations of European pine marten showed stress physiological response induced by the tourist pressure and this response is higher during reproductive season.

Keywords: Enzyme immunoassay; European pine marten; Faecal steroids; Stress; Tourist pressure

1. Introduction

The classical definition of term stress involves Physiological and neurophysiological changes in an organism [1]. Thus, metabolic, immunological and neuroendocrine mechanisms are involved in the description of stress reaction in physiological terms [2]. It is also well known that in vertebrates habitats are not static and animals have to be adapted to different internal and/or external stimuli known as stressors [3]. An adverse stimulus initiates a cascade of physiological responses and the activation of hypothalamus–pituitary–adrenal axis (HPA) with the result of both, glucocorticoid secretion (cortisol/corticosterone) by adrenal cortex [4,5], and the activation of sympatho-medullary system with the release of catecholamines [3]. Glucocorticoids have been used as physiological indicators of animal stress in a wide number of studies done in several species [2,5]. Short-term glucocortid secretion has been related to the adaptative response of animals to stressors and it is beneficial for the organism, they are involved in energy mobilisation and in behavioural changes. However, long-term glucocorticoid secretion may lead to some pathological status such as reproductive and/or endocrine disruption, suppression of the immune system, gastrointestinal ulcerations and much other adverse effects [5,6]. All these signs could significantly reduce both survival and reproductive success and without any doubt the species biological efficiency (fitness) [2,7].

Glucocorticoid measurement in animal species has been performed until recently by assaying plasma samples and is

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one of the accepted indexes of stress conditions [8,9]. However, plasma sample collection is an invasive method and requires the capture and/or manipulation of animals [10,11]. Wild carnivore species are a diverse group that shows different variations respect to their morphology, physiology and behaviour [11,12]. However, in natural environments all of them showed a common characteristic: they are species that show marked elusive behaviour and this represent extreme difficulty for being captured and the posterior manipulation induces stress responses during blood sampling procedures.

Recently, analysis of faecal steroid metabolits has been used as an accepted method for non-invasive evaluation of adrenocortical activity or a stress index of carnivore species and it has become an alternative to blood sampling [12,13]. Several authors have reported several advantages of faecal glucocorticoid measurements compared to blood. Faecal samples are easier to collect, it is not necessary the manipulation of animals [2,12,14,15]. This new technique has been applied for increasing number of carnivore species as African wild dogs [12], spotted hyenas [16], bears [10], wolves [17], black-footed ferrets [11,13], among others. However, most of studies have been performed in captivity and a few number of studies on environmental stress in natural conditions has been reported.

Human disturbances in the natural habitats may act as one of the stronger stressors in different species of wild animal populations [18–20]. Several reported studies performed in natural conditions, and mostly done in ungulates, e.g., white-tailed deer (*Odocoileus virginianus*) [21]; reindeer (*Rangifer tarandus*) [22]; moose (*Alces alces*) [23] but also in some carnivore species as wolf (*Canis lupus*) [24], have been related faecal glucocorticoid levels to human activities, mainly to sport activities concluding that higher levels of glucocorticoids were measured in those areas and times of the year when the use of snowmobiles was more intensive.

Finally, the information existed related to the endocrinology of European pine marten (*Martes martes*) is scarce, although this species is a popular carnivore in Europe included in the *Mustelidae* family. We only found two studies performed in black-footed ferret in which faecal glucocorticoid assays have been used as non-invasive method for monitoring adrenocortical activities and reproductive cycles in mustelids [11,13]. The possibility of performing such studies in the natural populations of European pine marten could be of outmost importance.

The aim of the study was to test the hypothesis that human tourist pressure could represent one of the main stressors in protected natural environments (parks) for the European pine marten (*Martes martes*). To test this hypothesis we will measure if the increase of faecal glucocorticoid concentrations is consequent to human disturbances. If the hypothesis is true we could predict that the faecal glucocorticoid concentrations should be higher in those times of the year and areas of the natural park that received the higher number of visitors.

2. Material and methods

2.1. Collection of faecal samples in the field

The faecal samples were collected during 15 months (from June 2004 to August 2005) at Natural Park Montes do Invernadeiro, an area of 5722 ha, located in Northwest Spain. In order to detect faecal samples of European pine marten several itineraries were established through the roads of the park and they were monthly surveyed on foot. The use of roads in European pine marten and other carnivores has been reported and these species frequently defecate on them, as a way of chemical communication [25–28].

The knowledge of European pine marten requirements in natural conditions is of greatest importance in order to establish the itineraries in the park. European pine marten is a carnivore species included in the family of mustelids, that shows solitary habits and defends individual territories (120–150 ha males, 90–110 ha females [29]. It becomes necessary to include the territory of several individuals to obtain an independent and representative data sample. Thus, the itineraries were established in four far away zones of Natural Park and in each the itineraries were separated by at least 1 km. Itineraries were conducted in optimal zones for the species by considering the previously obtained results on habitat selection and distribution of the species in the same study area [30].

Every time that a fresh scat (<12 h) of European pine marten was detected a sample was collected. For each sample the following data were registered: date of collection, estimated time between defecation and collection, and area of the park where the sample was collected. There is a discussion among authors referred to the stability of faecal samples and the possibility that environmental temperature conditions could vary faecal glucocorticoid concentrations [2,31,32] so we only collected fresh samples (<12 h between deposition and collection). Collection was favoured due to the nocturnal habits shown by this carnivore species and also by the fact that nocturnal environmental temperatures of the park are reasonable low during the whole year. Moreover scat age (fresh: deposition-12 h old; old: >13 h old) provided an estimate of the defecation time. Fresh faeces were characterised by a strong smell, a layer of mucus, and no signs of dehydration. Faecal hormone concentrations reflect that glucocorticoids have been secreted from adrenal cortex between 12 and 20/24 h before [20,33]. From each fresh scat almost a 10 g of sample was collected by means of a gloved hand. All collected samples were properly identified and maintained at −20 °C until assayed.

The natural park is divided in three areas: integral reservation (there is no tourist pressure, access is forbidden to visitors), restricted area or area of special protection (intermediate tourist pressure, visitors are allowed to enter always accompanied by the biologists of the park), free entry area or public-use area (high tourist pressure, all visitors are allowed to enter). Moreover in the free entry area there are several

lodges called "wildlife classrooms" where the visitor groups are allowed to spend the night. The number of visitors to the park was daily recorded.

It was considered that in the Natural Park coexisted two mustelid species: stone marten (*Martes foina*) and European pine marten (*Martes martes*). In order to discard the presence of stone marten in the study areas (it is not possible to differentiate faeces of this species from those of European pine marten) 38 fresh scats were randomly collected in the itineraries of the four defined park zones. Faecal DNA was assayed in each sample for the species identification by using the PCR-RFLPs technique [34]. Other identification methods such as camera-traps (155 camera-traps/night), visual surveys and detection of dead individuals were also used. Finally, European pine marten scats were differentiated from those of other sympatric carnivores (wildcat, red fox and wolf) by their size and shape.

2.2. Faecal extraction and enzyme immunoassays for steroid hormones

A total of 76 faecal samples of European pine marten were analysed for quantifying the faecal steroid hormone levels: glucocorticoids, androgens, estrogens and progestins.

2.2.1. Faecal steroid hormone extraction procedure

Extraction of steroid hormones from faecal samples was performed by using previous established methods for similar carnivore species and steroid hormones [11,35,36].

Frozen faecal samples were dried and pulverized, and then 0.2 g powered faeces were extracted by adding 5 ml of 90% ethanol, boiled during 20 min and then centrifuged (500 × *g*, 20 min). Supernatants were introduced into glass tubes and pellets were processed as previously described. The second supernatants were mixed with the firsts, dried under nitrogen air stream and then resuspended in 1 ml of 100% methanol that were maintained at −20 °C until assayed. The efficiency of extraction of each steroid hormone from faecal samples was tested by the addition of radiolabeled hormones (3H-cortisol, 3H-testosterone, 3H-estrone and 3H-progesterone, 4000–8000 dpm, ICN, CA, USA) to a parallel set of faecal samples prior to extraction.

2.2.2. Faecal steroid hormone enzyme immunoassays (EIAs)

Steroid hormones were analysed from faecal extracts by enzyme immunoassays developed in our laboratory and previously validated for the species, faecal extracts and particular hormone [37].

Polyclonal antibodies were raised in rabbits against cortisol-3CMO:BSA (C9130), testosterone-6CMO:BSA (C9003), progesterone-6CMO:BSA (C8912) and estrone 3-glucuronide:BSA (C9506). All antibodies were then purified and characterized for cross-reactivity against related steroid hormones. Hormone conjugates: Cortisol-3HS, testosterone-3HS, estrone-3HS and progesterone-3HS were labelled by horseradish peroxidase (Sigma, MO, USA). All steroids were obtained from Steraloids Inc. (Wilton, NH, USA).

Enzyme immunoassays were performed following the same assay protocol: 96 well flat bottomed polystyrene microtiter plates (Immulon 1B, Dynex, CA, USA) were coated with 100 μl/well of each purified antibody solution (1:4000 in coating buffer: sodium carbonate, 50 mM, pH 9.6) except for the first well which acted as plate/assay blank, and incubated overnight at 4 °C. Afterwards, non-bound antibodies were removed from the wells by washing plates five times with wash solution (NaCl, 150 M/l, Tween 20, 0.5 ml/l), inverted and dried.

Standards were solubilized in ethanol, evaporating the solvent and resolubilizing them in assay buffer (sodium phosphate, 100 mM, pH 7.0, with sodium chloride, 8.7 g/l, BSA, 1 g/l). Standard curve covered a range between 0 and 1 ng/well, and was constructed by using 10 standard solutions: 0.1, 0.5, 1, 5, 10, 50, 100, 500 and 1000 pg/well. The wells of the first and last rows were called B_0 (maximum binding of enzyme conjugate to the antibody), and 100 μl of conjugate dilutions (1:40,000 in assay buffer) were added to the wells. Standards and faecal extracts were analysed in duplicates. For standard curve each standard was resuspended in 150 μl of diluted conjugates, mixed and 50 μl were pipetted into the wells from the 2 and 6 rows. For faecal samples: 25 μl of each extract were evaporated and resuspended in 150 μl of diluted conjugates, and 50 μl were pipetted into the wells of 7–11 rows. Plates were covered and incubated for 2 h at room temperature. Bound/free separation was achieved by emptying plates by inversion and washing them, five times with wash solution. To evaluate the amount of labelled hormone bound to the antibody, 100 μl of substrate solution (3,3′,5,5′-tetramethylbenzidine dihydrochloride, pH 5.0, Neogen, (KY, USA) were added to all wells and incubated for 15 min at room temperature, this reaction was stopped by the addition of 100 μl of 1 M phosphoric acid. Absorbance was read at 450–600 nm in an automatic microplate reader.

Hormone concentrations were calculated by means of software developed for these techniques. Standard-dose response curve was constructed by plotting the binding percentage ($B/B_0 \times 100$) against hormone standard concentrations added. Faecal steroid hormone concentrations are expressed as ng/g dry faecal matter.

2.3. Statistical analysis

Statistical analysis was conducted with the software SPSS 9.0. Kolmogorov–Smirnov–Lilliefors test was used to determine the normal distribution of data. The faecal samples collected seasonally and in different areas of the park were considered as dependent data because the faecal samples collected in the itineraries surveyed seasonally corresponded to the same individuals, and in each area of the park the collected samples belonged to unknown individuals. In order to determine significant statistical differences

among k related samples Friedman's ANOVA test was used. Average data are presented as mean ± standard error (S.E.). The level of significance defined for statistical test was $p < 0.05$.

3. Results

The analysis of faecal DNA in 38 samples for the species identification showed that all analysed scats belonged to European pine marten. The other used methodologies also discarded the presence of stone marten in the study area, thus the specific origin samples and the obtained data reliability is guaranteed in the present study.

The mean recoveries of 3H-steroids from faecal extracts were as follows: 3H-cortisol: 81.5%, 3H-testosterone: 85.4%, 3H-estrone: 90.1% and 3H-progesterone: 85.5%.

Cross-reactivies of polyclonal antibodies were: for cortisol antibodies: cortisol 100%, prednisolone 9.9%, prednisone 6.3%, cortisone 5% and <1% with corticosterone, desoxycorticosterone, 21-desoxycortisone, testosterone, androstenedione, androsterone and 11-desoxycortisol. For testosterone antibodies: testosterone: 100%, 5-alpha-dyhidrotestosterone 20%, 5-beta-dyhidrotestosterone 5%, androstenedione 11.5%, androstenediol 3.5%, androstenelone 3.21%, 5-alpha-androstan-3-alpha, 17-beta diol 1% and <1% with cortisol, progesterone and estrone sulphate. For estrone sulphate: estrone sulphate 100%, estrone 100%, estrone glucuronide 100%, estradiol 2.12%, equilin 1.3% and <1% with equilenin, progesterone, testosterone and cortisol. For progesterone: progesterone 100%, 17-alpha-OH-progesterone 14.03%, 20-alpha-OH-progesterone 1.03%, 20-beta-OH-progesterone 1.46%, pregnenolone 2.73% and <1% with estradiol, estrone sulphate, testosterone and cortisol.

Parallel displacement curves were obtained for each hormone by comparing serial dilutions of pooled faecal extracts and the standard curves. Results showed that both curves were parallel in all cases.

Intra- and inter-assay coefficients of variation were calculated by assaying a pooled faecal sample 10 replicates in the same assay and the 10 replicates of the same sample in 10 consecutive assays: cortisol: 6.4% and 9.5.0%, testosterone: 5.5% and 8.9%, estrone sulphate 7.4% and 9.9%, progesterone 4.5% and 6.9%, respectively.

Assay sensitivities at maximum binding were the following: cortisol: 3.9 pg/well; testosterone: 4.9 pg/well, estrone-sulphate: 2.9 pg/well and progesterone: 2.5% pg/well.

The results showed that faecal cortisol metabolite concentrations were significantly lower in integral reservation area (15.95 ± 3.56 ng/g dry faeces) compared to restricted (31.4 ± 16.30 ng/g dry faeces) and free entry areas (41.59 ± 12.73 ng/g dry faeces), respectively (Fig. 1) ($p = 0.0001$ by Friedman's ANOVA) being faecal cortisol metabolite levels of European pine martens that occupied the restricted area intermediate between integral reservation and free entry areas.

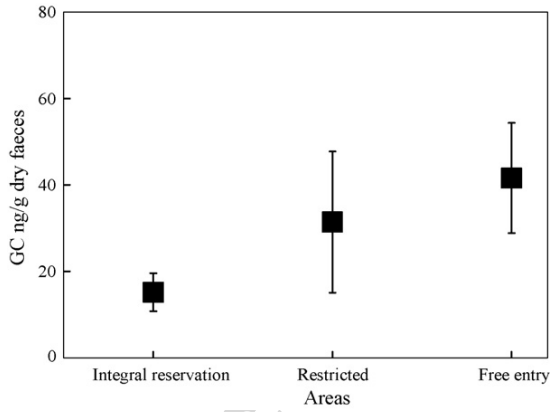

Fig. 1. Comparison of concentrations of faecal cortisol metabolites (mean ± S.E.) according to tourist pressure in different areas of the Natural Park.

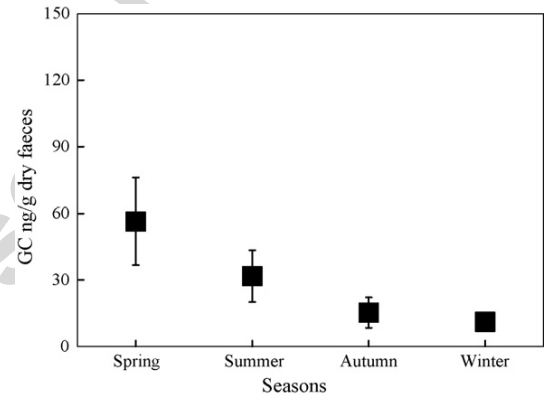

Fig. 2. Levels of faecal cortisol metabolites (mean ± S.E.) per season.

Faecal cortisol metabolite concentrations were significantly higher in spring (56.36 ± 19.62 ng/g dry faeces) and summer (31.27 ± 11.98 ng/g dry faeces) than in autumn (15.33 ± 6.89 ng/g dry faeces) and winter (11.13 ± 3.30 ng/g dry faeces) (Fig. 2) ($p = 0.0001$ by Friedman's ANOVA). These results are closely related to the daily visitor number that enter the park in each of the seasons analysed ($p = 0.001$ by Spearman's correlation). The highest number of visitors was recorded in spring (3204 visitors), followed by summer (1672 visitors), winter (646 visitors) and autumn (551 visitors) (Fig. 3).

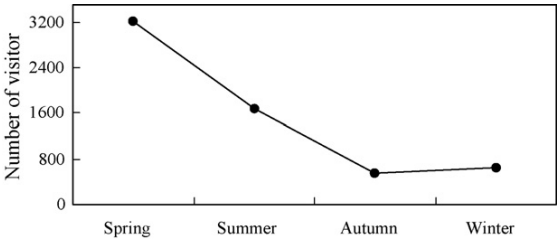

Fig. 3. Number of visitors in the Natural Park per season, $n = 6073$.

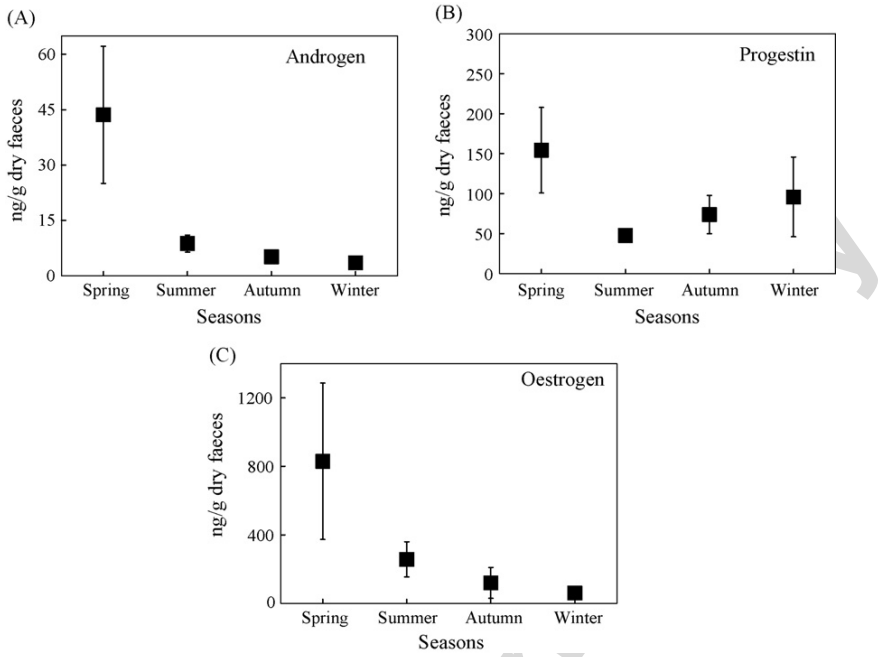

Fig. 4. Concentrations of faecal sexual hormones (A: testosterone, B: progesterone, C: estrone sulphate) per season (mean ± S.E.).

Faecal sex hormones (testosterone, progesterone and estrone sulphate) were significantly different among seasons of the year. Testosterone mean concentrations reached the highest levels at spring (reproductive season) (43.6 ± 18.6 ng/g dry faeces), followed by summer (7.5 ± 2.0 ng/g dry faeces), winter (3.5 ± 1.3 ng/g dry faeces) and autumn (2.5 ± 1.0 ng/g dry faeces) (Fig. 4A) ($p = 0.046$ by Friedman's ANOVA). Both progesterone and estrone sulphate also reached the highest levels during spring (P: 154.3 ± 53.5 ng/g dry faeces, E1S: 829.6 ± 456.1 ng/g dry faeces). Estrone sulphate showed decreasing levels in summer (257.2 ± 02.8 ng/g dry faeces), autumn (119.9 ± 89.4 ng/g dry faeces) and winter (60.8 ± 12.6 ng/g dry faeces), while progesterone faecal levels reached the minimum value in summer (47.6 ± 5.2 ng/g dry faeces) showing a progressive increase during autumn (73.9 ± 24.0 ng/g dry faeces) and winter (95.8 ± 49.9 ng/g dry faeces) (Fig. 4B and C). Both progesterone and estrone sulphate mean levels showed significant statistical differences between seasons (Progesterone $p = 0.0001$ and Estrone sulphate $p = 0.0001$ by Friedman's ANOVA, respectively).

4. Discussion

The present study shows that tourist pressure could be a source of disturbance in the European pine marten wild populations because stress hormone levels (glucocorticoids) increased significantly in those seasons (spring and summer) when the number of visitors in the natural environment of this species was higher.

Faecal hormone determinations have been used as non-invasive methods for monitoring adrenocortical and gonadal activities in wild species both maintained under captivity or in natural conditions [2,3,8,10–14,35,36,38]. However, limited information existed on the use of these techniques in mustelids. Recently, a cortisol EIA has been proved useful for non-invasively monitoring adrenocortical activity in the black-footed ferret [13]. The cortisol assay used in our study is similar to that used by these authors and obtained results in the European pine marten stated that it could be used for monitoring adrenocortical activity in this species. Respect to sex hormone determinations in the faeces of this species we though that the validated techniques are also suitable for monitoring reproductive status of the European pine marten. Similar techniques have been used in other mustelid species [35,36,38] showing similar results.

The effect of some human activities on the faecal glucocorticoid levels has been previously reported in different populations and species of wild animals [21–23]. In several animal species maintained under captivity a positive relationship between the number and the persistence of visitors to their facilities and physiological stress levels of the exhibited species has been also stated. In the present study, quantification of physiological stress response in terms of glucocorticoid secretion has been done by evaluating the tourist pressure that suffered the different areas of the Natural Park. Thus, higher glucocorticoid concentrations were measured in those areas of the park allowed to be visited by tourists (free entry zone and restricted area, respectively) when comparing to those with forbidden/restricted access (integral reservation area). These results clearly indicate the

concerns in the management of protected natural environments (parks and reservations) because it has been proved that it may be related to physiological stress responses that different species including the European pine marten exhibit. In the integral reservation area all collected samples showed higher variability among both individuals and values measured in these two areas – free entry and restricted area – that in turns they are signs of more intense response to physiological stress. This could be due to an unequal individual response to the same stressor of the European pine marten that are living in the same area. The present results showed that it is possible to detect incipient human disturbances in natural environments of wild species and this may help to adopt solutions on the management of these natural environments in order to minimize aversive interactions between humans and wild animal populations [24]. Physiological responses to human activities (tourism) may resulted in instantaneously responses (short-term stress) or being maintained during long time periods (chronic stress) that may interact to several variables directly related to biological efficiency (fitness) [6,7]. Therefore, long-term studies on the mentioned interactions between humans and wild animal populations similar to presented here are gaining increasingly relevance for avoiding chronic stress observed in wild populations that could reduce both reproductive success and species survival.

It has been pointed out higher standard mean deviations of faecal glucocorticoid concentrations were observed coincident to reproductive period – spring and summer – when compared to the rest of seasons. The explanation of these results could be due that the collected samples proceed not only from reproductive (breeding) individuals but also from non-reproductive ones, and as it has been measured for other species higher glucocorticoid levels are present in breeding individuals. These results were also consistent to those obtained for faecal sex steroid hormones measured (androgens, progestins and estrogens) showing also higher standard mean deviations during spring for the three sex hormone groups assayed. These data confirmed the hypothesis that faecal assayed samples not only arise from breeding individuals but also from sexually immature and old female and male individuals without breeding. The higher glucocorticoid levels observed during reproductive season (spring and summer) could also be related to reproductive behaviour that the species exhibit under natural conditions. This species copulates during summer time (July and August) and it is possible that one female breeds several males during the same breeding season. Implantation of embryos delayed approximately for 7 months and parturitions occur during next spring (April) after a period of 30 days of pregnancy [39]. Faecal glucocorticoid levels were higher during pregnancy and lactation periods (spring time) and then slightly lowered during heats (summer time). Similar results have been reported for other carnivore species such as spotted hyena where a significant increase in glucocorticoid secretion has been also observed during pregnancy and lactation periods [40]. Faecal sex hormone determinations (androgens, progestins and estrogens) revealed that in the European pine marten were significantly higher during breeding season (spring and summer) respect to the rest of the year. Monfort et al. [12] have reported significant elevations of faecal testosterone concentrations during breeding season for other carnivore species. They have also observed a significant rise in progesterone concentrations after copulation and during pregnancy being then lowered at parturition. Males of European pine marten may breed several females during heats (polygyny). Therefore, it is possible to expect strong competence among males in order to breed as much females as possible and this is followed by a rise in aggressive behaviour and higher levels of physiological stress. Another study performed in wolves resulted also in elevated physiological stress response during breeding season with higher levels of androgens measured for breeding males compared to non-breeders [20].

It has been concluded that wild populations of European pine marten seemed to be sensitive to human disturbances derived from tourist pressure reflected by increased glucocorticoid concentrations in those seasons and zones of their natural habitats where tourist pressure has shown to be more intensive. The presence of more visitors during spring and summer in the allowed areas of the Natural Park was coincident to the reproductive season of the European pine marten when the species has shown to be more sensitive to environmental disturbances so we could expect more intense physiological stress responses as it has been shown in this study during both seasons (spring and summer).

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DISTRIBUCIÓN Y HÁBITOS ALIMENTICIOS DE LA MARTA (*Martes martes*) EN EL PARQUE NATURAL OS MONTES DO INVERNADEIRO (GALICIA, NO DE ESPAÑA)

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Resumen

En este estudio se analizan la distribución y los hábitos alimenticios de la marta (*Martes martes* Linnaeus, 1758) en un área protegida del Macizo Central Ourensano (Galicia, NO España). Para evaluar la presencia de la especie se efectuaron transectos a pie en 67 cuadriculas UTM (1 km^2) del Parque Natural Os Montes do Invernadeiro en busca de sus excrementos. Además, se usaron otras metodologías complementarias como el fototrampeo, la observación directa y la localización de ejemplares muertos. Para estudiar la dieta se analizaron 209 excrementos recolectados en 25 transectos recorridos mensualmente entre julio 2004 y junio 2005. Para discriminar los excrementos de marta de los de garduña (*Martes foina*) se usaron técnicas moleculares (PCR-RLFPs), siendo la primera vez que se usa esta metodología en un estudio sobre la alimentación de la especie. Los resultados indican que la marta ocupa el 32,8% del área estudiada. Los mamíferos constituyeron la base principal de su dieta todo el año (50,0% de la biomasa ingerida), seguidos por los frutos (28,1%), aves (20,9%), insectos (0,8%) y reptiles (0,2%). Los micromamíferos constituyeron la especie presa más consumida (41,6% de la biomasa total ingerida), destacando principalmente el consumo de especies del género *Apodemus* (19,1%). En primavera e invierno los mamíferos fueron la presa predominante (65,8% y 79,5% respectivamente). Sin embargo, en verano y otoño el alimento más consumido fueron los frutos de serbal (*Sorbus aucuparia*) (verano: 49,7%, otoño: 59,9%), seguidos por los mamíferos (verano: 27,2%, otoño: 30,9%).

Palabras clave: dieta, distribución, España, excrementos, marta.

Abstract

*Distribution and feeding habits of the Pine marten (*Martes martes*) at Os Montes do Invernadeiro Natural Park (Galicia, NW Spain)*

The distribution and feeding habits of the Pine marten (*Martes martes* Linnaeus, 1758) were studied in a protected area of Macizo Central Ourensano (Galicia, NW Spain). To evaluate its presence, two observers surveyed transects on foot. The transects were carried out on 67 grids (UTM 1 km^2) of Os Montes do Invernadeiro Natural Park to detect pine marten faeces.

Other complementary methodologies, such as camera-traps, visual surveys and detection of dead individuals, were also used. To study feeding habits, 209 faeces were analysed and collected in 25 transects surveyed monthly between July 2004 and June 2005. Analysis of faecal DNA was used for specific identification, using the PCR-RFLPs technique. This methodology was used for the first time in a study on pine marten diet. The results indicate that the species occupies 32.8% of the studied area. Mammals constituted the main prey all year round (50.0% ingested biomass), followed by fruit (28.1%), birds (20.9%), insects (0.8%) and reptiles (0.2%). Small mammals formed the most depredated prey species (41.6% ingested biomass), mainly *Apodemus* species (19.1%). Mammals were among the most-consumed prey in spring and winter (65.8% and 79.5%, respectively). However, in summer and autumn the species fed mainly on rowanberry fruit (*Sorbus aucuparia*) (summer: 49.7%, autumn: 59.9%), followed by mammals (summer: 27.2%, autumn: 30.9%).

Key words: diet, distribution, faeces, Pine marten, Spain.

Introducción

En España la marta (*Martes martes* Linnaeus, 1758) tiene una distribución principalmente Eurosiberiana, ocupando la franja norte (cornisa cantábrica, franja pirenaica y Galicia) y también las islas de Mallorca y Menorca (Barea y Ballesteros 1999, Palomo y Gisbert 2002). En Cataluña, Aragón y Navarra se distribuye por los Pirineos de manera uniforme, ocupando áreas forestales (Ruiz-Olmo *et al.* 1988).

El hábitat que ocupa la marta se caracteriza por la presencia de bosques eurosiberianos de coníferas y bosques caducifolios (Delibes 1983, Mitchell-Jones *et al.* 1999). En el Parque Natural Os Montes do Invernadeiro la presencia de la especie está relacionada con los bosques caducifolios y con los bosques maduros de pino albar (*Pinus sylvestris*), seleccionando la marta además positivamente las zonas cercanas a los cursos de agua y con una proporción importante de roca (Barja 2005a).

En Europa la dieta de la marta está compuesta principalmente por micromamíferos, destacando roedores de los géneros *Clethrionomys*, *Microtus* y *Apodemus* (Clevenger 1993a, 1994, De Marinis y Masseti 1995) que aparecieron en al menos el 50% de los excrementos analizados (Marchesi y Mermod 1989, Zalewski *et al.* 1995). Los frutos, insectos y aves también adquieren gran relevancia en su dieta, especialmente en verano y otoño (Marchesi y Mermod 1989, Clevenger 1993a, Ruiz-Olmo y López-Martín 1996).

En la Península Ibérica la marta es un carnívoro escasamente conocido, no existiendo datos exhaustivos sobre distribución, ni sobre el estado actual de las poblaciones (Palomo y Gisbert 2002). Hasta la fecha se han publicado pocos trabajos en revistas científicas sobre los hábitos alimenticios de la especie en la franja norte de la Península Ibérica, habiendo sido realizados la mayoría en las Islas Baleares y sólo unos pocos abarcan la totalidad del año (Braña y del Campo 1982, Moreno *et al.* 1988, Ruiz-Olmo y Nadal 1991, Clevenger 1993a, b, 1994, Ruiz-Olmo y López-Martín 1996).

Los objetivos del presente trabajo fueron delimitar el área de distribución de la marta en el Parque Natural Os Montes do Invernadeiro (SE de Ourense) y estudiar sus hábitos alimenticios a lo largo del año.

Material y métodos

Área de estudio

El área de estudio ocupa 5.722 ha, incluyendo el Parque Natural Os Montes do Invernadeiro que se sitúa en el Macizo Central Ourensano, al noroeste de la Península Ibérica. El paisaje se caracteriza por una topografía accidentada y la altitud oscila entre los 880 m y los 1.707 m (Barja 2001). El clima tiene una tendencia a la continentalidad combinando influencias atlánticas y mediterráneas. Los montes del Invernadeiro cuentan con una flora variada, alternando comunidades vegetales mediterráneas con bosques relictos atlánticos (Castroviejo 1977). La vegetación predominante está constituida por matorrales mixtos de brezo (*Erica australis*), carquesia (*Pterospartum tridentatum*) y jaguarzo (*Halimium lasianthum*). En los valles y vaguadas predomina el bosque caducifolio autóctono formado principalmente por asociaciones de roble (*Quercus robur*), abedul (*Betula celtiberica*), acebo (*Ilex aquifolium*) y serbal (*Sorbus aucuparia*). Extensas áreas del parque están ocupadas por repoblaciones de pino albar (*P. sylvestris*) (Barja 2001).

Uso de varias metodologías para descartar la presencia de garduña

Para cerciorarse de que todos los excrementos detectados y recolectados durante el estudio eran de marta y no se estaban incluyendo excrementos de garduña, se analizaron mediante métodos moleculares 38 excrementos fres-

cos localizados por toda el área de estudio, utilizando la técnica PCR-RFLPs (Berdión y Gómez-Moliner 2004, Gómez-Moliner *et al.* 2004). Asimismo, la presencia de la garduña en el área de estudio fue descartada también mediante otras metodologías: fototrampeo (155 cámaras trampa/noche), observación directa y detección de cadáveres. Durante los recorridos por el área de estudio se anotaron todos los avistamientos y localización de individuos muertos. Los datos obtenidos con estos métodos también fueron usados para determinar la presencia de la especie.

Búsqueda y recolección de excrementos

Para delimitar el área de distribución de la marta en el parque, en noviembre de 2004 se recorrieron a pie transectos de 600 m en cada una de las 67 cuadriculas UTM de 1 km^2 del área de estudio. Los transectos fueron realizados en la zona que parecía reunir las mejores condiciones para la presencia de la especie y se anotaron todos los excrementos detectados durante los recorridos. En total se recorrieron 40,2 km por pistas forestales, por ser éstas usadas con frecuencia por la marta en sus desplazamientos y depositar en ellas sus heces como parte del marcaje oloroso (Barja 2005b), al igual que ocurre en otros carnívoros (Robinson y Delibes 1988, Barja *et al.* 2004, 2005). Todos los recorridos fueron realizados por 2 observadores.

La recolección de excrementos para estudiar la dieta duró un año (julio 2004 - junio 2005). Considerando los resultados obtenidos en estudios previos realizados en el área de estudio sobre los requerimientos de la especie (Barja 2005a, b) y el área de distribución obtenida en este estudio, todos los meses se recorrieron a pie por pistas forestales 25 transectos de 300 m cada uno (7,5 km recorridos/mes). Los recorridos mensuales se realizaron a final de mes para evitar asignar erróneamente el mes de deposición y poder analizar las variaciones estacionales en la dieta. Para no duplicar la información cada mes que se recorrían los transectos sólo se anotaban los excrementos que fueron depositados después del muestreo mensual precedente. Para el análisis estacional de la dieta los meses se agruparon de la siguiente manera: primavera (abril-junio), verano (julio-septiembre), otoño (octubre-diciembre) e invierno (enero-marzo).

Para que la muestra de excrementos recolectada fuera representativa e incluyera los territorios de varias martas, los transectos se realizaron en cuatro zonas

del parque alejadas entre sí. Como el territorio de las martas en Europa es de 1,5-2,2 km^2 (Zalewski *et al.* 1995), para evitar la dependencia de los datos, dentro de cada zona los transectos fueron separados 700 m.

Análisis de los excrementos

Los excrementos recolectados se limpiaron en el laboratorio siguiendo el procedimiento convencional (Reynolds y Aebischer 1991). Una vez que éstos estaban secos se separaron los diferentes componentes macroscópicos, atribuyendo cada uno a un grupo taxonómico en función de sus características (mamíferos: presencia de pelo y presencia de huesos; aves: presencia de plumas y huesos; reptiles: presencia de escamas; insectos: presencia de exoesqueletos, cutículas de larvas y capullos; frutos: presencia de semillas y cáscaras). Los diferentes componentes fueron pesados por separado con una báscula de precisión para estimar sus importancias relativas en la dieta de la marta. Para identificar las diferentes especies de mamíferos ingeridas, como apenas aparecían mandíbulas, se realizaron improntas de la cutícula de los pelos y análisis de la médula de los mismos. A partir de las improntas de la cutícula y de las médulas de los pelos analizados se identificó la especie presa comparando con los atlas de identificación de pelos de mamíferos (Faliu *et al.* 1980, Teerink 1991). Además, los pelos que aparecían en los excrementos fueron comparados con el pelaje de las diferentes especies de micromamíferos que alberga el Museo Nacional de Ciencias Naturales (CSIC, Madrid), algunos de los cuales fueron recolectados en el área de estudio. Por tanto, los patrones de coloración de los pelos, su longitud y grosor y las improntas de la cutícula y análisis de la médula permitió la discriminación de los diferentes mamíferos presa.

Análisis de los datos

Para calcular la biomasa ingerida de las diferentes categorías de presas se utilizaron los factores de corrección de Lockie (1960). Como los datos no se ajustaron a una distribución normal se emplearon pruebas estadísticas no paramétricas. Para comprobar si las diferencias entre las frecuencias observadas y las esperadas son significativas se usó el test de la χ^2. Las Tablas de Contingencia se usaron para contrastar la independencia de las variables de las tablas. Se rechazó la hipótesis nula cuando $p < 0,05$. Los análisis estadísticos se llevaron a cabo con el software SPSS 9.0.

Resultados

Distribución de la especie

El análisis molecular de 38 excrementos frescos recolectados puso de manifiesto que todas las muestras corresponden a haplotipos de marta. Además, las 22 fotografías obtenidas, la observación de 4 animales y la localización de 1 ejemplar muerto constataron solamente la presencia de marta, no detectándose con ninguna de las metodologías la presencia de garduña en el área de estudio.

Las diferentes metodologías empleadas detectaron la presencia de la marta en 22 cuadrículas de las 67 prospectadas (32,8% del área estudiada) (Figura 1). En 21 cuadrículas (95,5% de las cuadrículas con presencia) se encontraron excrementos, en 5 (22,7%) se fotografiaron un total de 4 ejemplares diferentes, en 3 (13,6%) se efectuaron avistamientos de 4 individuos y en 1 (4,5%) se encontró el cadáver de un macho adulto. La garduña no fue detectada en ninguna de las cuadrículas del área de estudio.

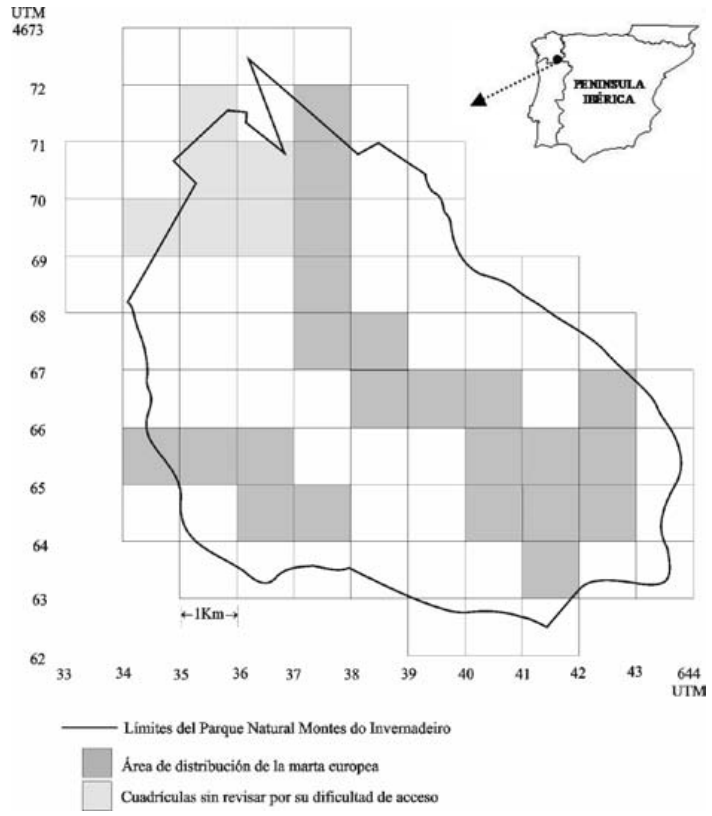

Figura 1. Distribución de la marta en el área de estudio.

Distribution of Pine marten in the study area.

Hábitos alimenticios

El número de excrementos analizados para estudiar la dieta fue de 209 (verano: 57, otoño: 54, invierno: 32 y primavera: 66). El 2,9% de los mismos contenía sólo materia indiferenciada (n= 6) y el 2,4% sólo hojas secas (n= 5). Para calcular la biomasa ingerida estos excrementos fueron desestimados de la muestra (n= 11), y se consideraron 198 excrementos. El número mínimo de presas por excremento osciló entre 1 y 3, con una media de 1,31 ± 0,53. El 72,8% de los excrementos contenía restos de una presa, el 23,9% de dos y el 3,3% de tres.

Los mamíferos constituyeron la base de la dieta de la marta en el área de estudio representando el 50,0% de la biomasa ingerida a lo largo del año, seguidos por frutos (28,1%), aves (20,9%), insectos (0,8%) y reptiles (0,2%) (Tabla 1) (χ^2= 180,3; g.l.= 4; p< 0,001; n= 273). Dentro de los mamíferos, los roedores fueron consumidos con una frecuencia significativamente mayor que los insectívoros y la carroña (65,1% de la biomasa ingerida, 22,2%, 12,7% respectivamente) (Tabla 1) (χ^2= 67,6; g.l.= 2; p< 0,001; n= 121). Las carroñas consumidas eran de corzo (66,6% de la biomasa), jabalí (26,6%) y tejón (6,8%). Los micromamíferos constituyeron la presa principal (41,6% de la biomasa ingerida), principalmente el género *Apodemus* (19,1%). Los roedores depredados por la marta fueron: *Apodemus* sp. (58,7% de la biomasa ingerida dentro del orden), lirón gris (*Glis glis*) (13,1%), topillo rojo (*Clethrionomys glareolus*) (8,9%), ardilla roja (*Sciurus vulgaris*) (6,4%), *Arvicola* sp. (5,2%), lirón careto (*Eliomys quercinus*) (3,4%), *Microtus* sp. (2,8%) y *Mus* sp. (1,5%) (Tabla 1). Las diferencias en el consumo de los distintos roedores resultaron significativas (χ^2= 189,8; g.l.= 7; p< 0,001; n= 85). Entre los insectívoros, el topo ibérico (*Talpa occidentalis*) supuso el 28,9% de la biomasa ingerida, *Crocidura* sp. el 24,6%, el musgaño patiblanco (*Neomys fodiens*) el 24,6% y *Sorex* sp. el 21,9% (Tabla 1). Las diferencias en el consumo de los insectívoros no resultaron significativas (χ^2= 1,0; g.l.= 3; p>0,05; n= 24).

Los frutos del serbal (*S. aucuparia*) constituyeron el 95,8% de la biomasa vegetal ingerida, los de arraclán (*Frangula alnus*) el 2,6% y los de acebo (*I. aquifolium*) el 1,6% (Tabla 1). Las diferencias en la ingestión de frutos de las diferentes especies fueron significativas (χ^2= 120,0; g.l.= 2; p< 0,001; n= 77).

Las aves depredadas pertenecían a los órdenes de las Paseriformes y Piciformes (20,9% biomasa ingerida). Los insectos consumidos pertenecían a distintos órdenes: Coleóptera (42,3% de la biomasa ingerida), Lepidóptera (42,1%), Heteróptera (14,2%) y mudas de especies sin identificar (1,4%). Todos los reptiles consumidos pertenecían a la familia de los lacértidos (Tabla 1).

TABLA 1
Dieta anual de la marta desde julio de 2004 hasta junio de 2005.

Annual diet of Pine marten from July 2004 to June 2005.

		Especie/Género	**FA** (n= 209)	**% A** (n= 209)	**BI**[1] (n= 198)	**% BI** (n= 198)
Mamíferos	Roedores	*Sciurus vulgaris*	5	2,4	105,4	2,1
		Apodemus sp.	52	24,9	956,8	19,1
		Mus sp.	1	0,5	25,1	0,5
		Clethrionomys glareolus	7	3,3	145,0	2,9
		Arvicola sp.	4	1,9	87,1	1,7
		Microtus sp.	2	1,0	45,1	0,9
		Eliomys quercinus	4	1,9	54,1	1,1
		Glis glis	10	4,8	214,5	4,3
	Insectívoros	*Sorex* sp.	7	3,3	116,2	2,3
		Neomys fodiens	6	2,9	130,5	2,6
		Crocidura sp.	7	3,3	132,3	2,6
		Talpa occidentalis	4	1,9	151,8	3,0
		Insectívoro no identificado	2	1,0	23,3	0,5
	Ungulados	*Capreolus capreolus*	5	2,4	210,8	4,2
		Sus scrofa	4	1,9	84,2	1,7
	Carnívoros	*Meles meles*	1	0,5	21,4	0,4
		Total mamíferos	**117**	**56,0**	**2503,6**	**50,0**
Aves	Paseriformes y Piciformes		32	15,3	1043,0	20,9
		Total aves	**32**	**15,3**	**1043,0**	**20,9**
Reptiles	Lacertidos		3	1,4	9,0	0,2
		Total reptiles	**3**	**1,4**	**9,0**	**0,2**
Insectos	Coleópteros		12	5,7	17,6	0,4
	Lepidópteros		3	1,4	17,5	0,3
	Heterópteros		2	1,0	5,9	0,1
	Muda no identificada		2	1,0	0,6	-
		Total insectos	**19**	**9,1**	**41,6**	**0,8**
Vegetales	Rosácea	*Sorbus aucuparia*	71	34,0	1348,1	26,9
	Aquifoliácea	*Ilex aquifolium*	4	1,9	25,5	0,5
	Rhamnácea	*Frangula alnus*	2	1,0	34,2	0,7
	Hojas		8	3,8	-	-
		Total vegetales	**86**	**41,1**	**1407,8**	**28,1**
Materia indiferenciada			10	2,9	-	-

FA: Frecuencia de aparición= n° de excrementos en los que aparece cada tipo de alimento.
% A: Porcentaje de aparición= (FA / n° total de excrementos) x 100.
BI: Biomasa ingerida de un tipo de presa = gramos de presa ingerida.
% BI: Porcentaje de biomasa ingerida de un tipo de presa = (BI / BI total) x 100.
[1] para calcular la biomasa ingerida no se han considerado los excrementos que estaban constituidos sólo por materia indiferenciada y hojas secas (n= 11).

El consumo de los diferentes tipos de alimento (mamíferos, frutos, aves, insectos, reptiles) varió estacionalmente (Pruebas Exactas de Monte Carlo: χ^2= 58,4; g.l.= 9; p< 0,001; n= 252). En primavera los mamíferos constituyeron la presa principal, suponiendo el 65,8% de la biomasa ingerida (roedores: 36,4%, insectívoros: 18,9%, carroñas: 10,5%), seguidos por aves (31,7%), insectos (1,9%) y reptiles (0,6%) (Figura 2). Las diferencias en las frecuencias de consumo de los distintos tipos de presas en primavera resultaron significativas (χ^2= 43,8; g.l.= 3; p< 0,001; n= 72). En verano las bayas de serbal fueron el alimento seleccionado (49,7% de la biomasa consumida). En esta estación disminuyó la presión de depredación sobre las diferentes especies de mamíferos, suponiendo éstos el 27,2% de la biomasa ingerida (roedores: 21,6%, insectívoros: 5,6%) y constituyendo el segundo tipo de alimento más importante. En verano las aves supusieron el 22,1% de la biomasa ingerida y los insectos sólo el 1%. Durante esta estación la marta no consumió carroñas ni tampoco depredó sobre los reptiles (Figura 2). Las diferencias en las frecuencias de consumo de los diferentes tipos de alimento resultaron significativas (χ^2= 33,4; g.l.= 3; p< 0,001; n= 67). En otoño, al igual que en verano, los frutos de serbal constituyeron el principal alimento (59,9% de la biomasa ingerida), seguidos por los mamíferos (30,9% de

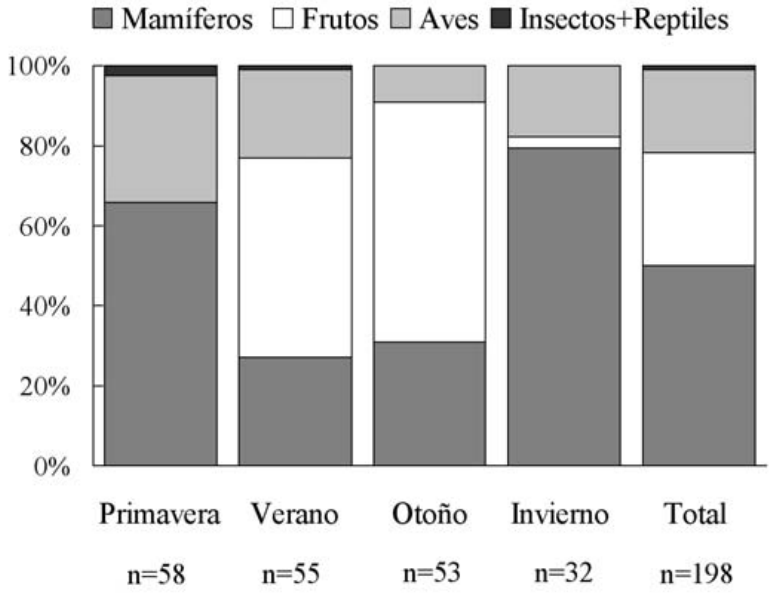

Figura 2. Variación estacional en la dieta de la marta en términos de biomasa ingerida.

Seasonal variation in Pine marten's diet in terms of ingested biomass.

la biomasa ingerida; roedores: 26,6%, insectívoros: 4,2%, carroñas: 0,1%). Las aves supusieron el 9,2% de la biomasa ingerida y los reptiles e insectos no estaban representados en la dieta otoñal (Figura 2) (χ^2= 30,8; g.l.= 2; p< 0,001; n= 74). En invierno los mamíferos fueron de nuevo la presa principal y alcanzaron en esta estación su mayor representación en la dieta de la marta (79,5% de la biomasa ingerida; roedores: 49,2%, insectívoros: 14,3%, carroñas: 16%). Las aves supusieron el 17,7%, los frutos el 2,7% y los insectos el 0,1% (Figura 2) (χ^2= 45,8; g.l.= 3; p< 0,001; n= 39).

Discusión

Los análisis genéticos confirmaron el fenómeno de desplazamiento ecológico de la marta hacia la garduña a pequeña escala. En zonas de simpatría la marta parece ocupar las zonas más forestales mejor conservadas y la garduña quedar relegada a áreas más humanizadas (Delibes 1983). Cabe destacar que éste es el primer estudio realizado sobre distribución y hábitos alimenticios de la marta en el cual se han usado técnicas moleculares para descartar la presencia de garduña en el área de estudio. Esto sin duda garantiza la procedencia de los excrementos analizados y la fiabilidad de los resultados obtenidos. En el área de estudio la marta está presente en las cuadrículas que presentan mayor proporción de bosques caducifolios, pinares maduros y roquedos, así como cursos permanentes de agua (Barja 2005a).

En la dieta de la marta en el área de estudio predominaron los mamíferos, coindiciendo estos resultados con los obtenidos en otros países europeos (Pulliainen 1980, Marchesi y Mermod 1989, Jedrzejewski *et al.* 1993, Zalewski *et al.* 1995) y con los obtenidos por Clevenger (1993a) para la Cordillera Cantábrica. Por el contrario, en otros trabajos llevados a cabo en la Cordillera Cantábrica y en Pirineos se puso de manifiesto que los frutos constituían el alimento principal todo el año (Braña y Del Campo 1982, Guitián y Callejo 1983, Ruiz-Olmo y López-Martín 1996). En Menorca los mamíferos, aves, frutos e insectos tuvieron importancias relativas similares (Clevenger 1993a, b). El hecho de que dentro de la categoría de los mamíferos fueran los roedores el grupo más depredado también coincidió con los resultados de otros estudios realizados en Europa (Pulliainen 1980, Marchesi y Mermod 1989, Jedrzejewski *et al.* 1993, Zalewski *et al.*

1995). Al igual que en la Cordillera Cantábrica (Clevenger 1993a), en el Parque Natural Os Montes do Invernadeiro el género *Apodemus* resultó ser el más consumido dentro de los mamíferos. Sin embargo, en el Parque Nacional de Bialowieza en Polonia, en la Jura Suiza y en Finlandia prevaleció el género *Clethrionomys* (Pulliainen 1980, Marchesi y Mermod 1989, Jedrzejewski *et al.* 1993, Zalewski *et al.* 1995). En nuestra área de estudio la marta consumió principalmente serbal, contrariamente a lo que ocurrió en la Cordillera Cantábrica y en la región boreal donde predominó el género *Vaccinum* (Pulliainen 1980, Braña y Del Campo 1982). En Suiza predominó *Rosa* sp. (Marchesi y Mermod 1989) y en Menorca la especie consumió principalmente moras (*Rubus ulmifolius*), higos (*Ficus carica*) y algarrobos (*Ceratonia siliqua*) (Clevenger 1993b).

Las aves, las terceras en importancia después de los mamíferos y frutos, fueron consumidas a lo largo de todo el año, coincidiendo estos resultados con los obtenidos para las poblaciones estudiadas en Suiza y en Menorca (Marchesi y Mermod 1989, Clevenger 1993a). El consumo de reptiles e insectos es ocasional en el área de estudio. También en Polonia ambas categorías tuvieron una escasa representación en la dieta de la marta (Jedrzejewski *et al.* 1993). En la población de Menorca los insectos tuvieron una importancia similar a los mamíferos, aves y frutos (Clevenger 1993a). En la Jura Suiza también los insectos fueron consumidos con una frecuencia considerable (Marchesi y Mermod 1989). Los resultados obtenidos en el área de estudio podrían deberse al carácter continental del clima que puede determinar una baja disponibilidad de insectos.

La dieta primaveral de la marta en el Parque Natural Os Montes do Invernadeiro, con predominio de roedores y aves, coincidió con lo observado en Polonia por Jedrzejewski *et al.* (1993) y en Menorca por Clevenger (1993a, b). En Suiza fueron más consumidos los mamíferos y frutos en esta estación (Marchesi y Mermod 1989); mientras que en la Cordillera Cantábrica las presas seleccionadas fueron los mamíferos y los insectos (Clevenger 1993a). El hecho de que las aves fueran depredadas preferentemente en esta estación puede guardar relación con la facilidad de captura de los polluelos debido a su carácter indefenso y a la mayor accesibilidad a los nidos. El hecho de que en verano fueran los frutos el alimento dominante coincidió con la alta disponibilidad de bayas maduras de serbal a partir de finales de agosto. En esta estación las bayas de serbal están disponibles en las ramas de los árboles, y a partir del mes de septiembre es cuando caen al

suelo (Rosellini y Barja obs. pers.). Sin embargo, la alta frecuencia de consumo en verano nos habla acerca del carácter arborícola de la especie, lo que le permite tener acceso a este tipo de alimento durante esta estación. También en otoño las bayas de serbal fueron el alimento predominante en la dieta, coincidiendo estos resultados con los de la Cordillera Cantábrica (Guitián y Callejo 1983, Clevenger 1993a, Bermejo y Guitián 1996), Pirineos (Ruiz-Olmo y López-Martín 1996), Suiza (Marchesi y Mermod 1989) y Menorca (Clevenger 1993a). Esto parece indicar de nuevo la importancia de la facilidad de captura del alimento; así la especie consume los frutos de serbal (con bajo poder nutricional) a pesar de disponer en el medio de otras presas que le aportarían más energía, pero para las cuales presumiblemente se requiere invertir más esfuerzo en su captura. En invierno los carnívoros de tamaño medio tienen menor facilidad de acceso a los recursos tróficos, sobre todo en el área de estudio donde el terreno permanece buena parte del tiempo cubierto de nieve. En esta estación la marta, aunque consume preferentemente micromamíferos, aprovecha para alimentarse también de carroñas de ungulados, matadas por los lobos que ocupan la zona. Así, aparecieron en sus excrementos restos de corzo y jabalí, las dos presas principales del lobo en el área de estudio (Barja datos sin publicar). Este comportamiento de la marta fue también observado en el Parque Nacional de Bialowieza en Polonia y fue interpretado como una estrategia evolutiva para evitar la transmisión de zoonosis al alimentarse de animales muertos por enfermedades propias (Jedrzejewski *et al.* 1993, Zalewski *et al.* 1995).

Dentro de los mamíferos, la marta depredó preferentemente sobre el ratón de campo a lo largo de todo el año, la especie presa más abundante en todos los hábitats del área de estudio (Rosellini y Barja datos sin publicar), resultando un recurso básico y fundamental en su dieta. El hecho de que el ratón de campo junto con el lirón gris (las dos especies presa más importantes) sean animales básicamente forestales, pone de manifiesto el uso preferencial de este tipo de hábitat por la marta en el área de estudio, como han indicado estudios previos en la zona (Barja 2005a) y en otras áreas de Europa (O'Sullivan 1983, Brainerd 1990).

La presencia del lirón gris en la dieta de la marta ocurrió principalmente en primavera, cuando este animal se despierta del letargo invernal y se encuentra más débil y vulnerable. La familia Soricidae, la segunda en importancia dentro de la categoría de los mamíferos, sufrió una tasa de depredación por parte de la

especie similar a lo largo de todo el año. Los insectívoros no suelen sufrir altas tasas de depredación por parte de los carnívoros debido a su sabor desagradable (Dickman y Doncaster 1984). Sin embargo, para la marta los sorícidos constituyeron un tipo de recurso aprovechado durante todo el año. Cabe destacar que el género *Crocidura* había sido citado hasta ahora en la dieta de la marta únicamente en un estudio realizado en la Cordillera Cantábrica (Clevenger 1993a). La presencia del musgaño patiblanco parece indicativo además de la búsqueda de presas también en ambientes ribereños y/o encharcados. La ardilla roja no es una presa fácil para el mustélido (Noblet 2002), y fue depredada sobretodo en verano, cuando los jóvenes son más abundantes y seguramente más fáciles de atrapar. En la dieta de la marta en el área de estudio resulta también interesante la aparición del topo ibérico, por ser este animal fosor, lo cual dificulta enormemente su captura y requiere una búsqueda activa.

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Regular research paper

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THE RESPONSE OF EUROPEAN PINE MARTEN (*MARTES MARTES* L.) FEEDING TO THE CHANGES OF SMALL MAMMAL ABUNDANCE

ABSTRACT: The European pine marten (*Martes martes*) is commonly classified as an opportunistic predator. If this is the case, the species ought to show seasonal differences in the small mammal composition of its scats – the types of prey taken depending on their abundanc. In addition, it ought to consume the food that requires lower energy cost for their acquisition in each season. The feeding strategy of the European pine marten was studied in northwestern Spain by analyzing 209 scats collected between July 2004 and June 2005, and by seasonally trapping small mammals to obtain information on their abundance. The study area (5,722 ha) was located in a mountainous region (1,707–880 m a.s.l.) and covered with brushwood and deciduous forest (oak, birch, holly and pine). Molecular analysis of scats (PCR-RFLP) was performed to rule out the presence of the stone marten (*Martes foina* L.). The frequency of occurrence and biomass of the small mammals (the main prey species) preyed upon each season were compared. The pine marten consumed significantly more small mammals in the seasons in which their abundance was the lowest (winter and spring). In the autumn, when the highest number of small mammals was detected, the pine marten did not increase its predation of them. These results indicate that the European pine marten is not an opportunistic predator. Rather, the feeding strategy adopted by the species seems to be intermediate between that of an opportunist and specialist predator.

KEY WORDS: European pine marten, abundance, opportunist, scats, small mammals, specialist

1. INTRODUCTION

In Europe, the diet of the European pine marten (*Martes martes* L., 1758) is varied, and includes mammals, fruits, birds and insects (De Marinis and Masseti 1995), although small mammals are the year-round main prey items (Marchesi and Mermod 1989, Zalewski *et al*. 1995). Fruits, birds and insects are consumed preferably in autumn, spring, summer (Marchesi and Mermod 1989, Clevenger 1993, Jędrzejewski *et al*. 1993). In Scandinavia the European pine marten takes squirrels (*Sciurus vulgaris* L., 1758) (Storch *et al*. 1990) and hares (*Lepus* sp.) (Helldin 1999) when small mammals are scarce. In Poland, carrion (mainly roe deer *Capreolus capreolus* L., 1758) and birds' eggs are important during the spring (Zalewski *et al*. 1995). In the Balearic Islands, garbage seems to provide an alternative food source during periods of prey shortage (Moreno *et al*. 1988).

The European pine marten is commonly defined as an opportunist predator since its

diet is very varied (Marchesi and Mermod 1989, Clevenger 1994, Helldin 1999). However, it is important to notice that the latter authors did not analyze prey abundance extensively. The only study to date that suggests a certain degree of predatory specialization on the part of this species was performed at Bialowieza National Park (Poland), where the bank vole (*Clethrionomys glareolus* Schreber, 1780) was the main prey, even though the yellow-necked mouse (*Apodemus flavicollis* Melchior, 1834) is the most abundant species in the study area (Jędrzejewski *et al.* 1993, Zalewski *et al.* 1995).

A species is considered a trophic specialist when it feeds almost entirely on one prey species, and when it shows this preference regardless of the prey's availability. Such predators are said to show a type II (hyperbolic) functional response (Holling 1959). An opportunist, however, consumes the food that is most available in each season and area, changing its diet depending on food availability. When the abundance of one prey type diminishes, opportunist predators begin to take a more abundant species; they therefore show a type III functional response (S-shaped) (Holling 1959, Glasser 1982, Angelstam *et al.* 1984, Erlinge 1986, Futyma and Moreno 1988).

The aim of the present study was to test the hypothesis that the European pine marten is an opportunist species. If it is an opportunist it ought to show significant seasonal differences in the consumption of small mammals (its main prey), changing what it takes according to abundance. An increase in small mammal consumption would be expected in seasons in which such prey is more abundant. In addition, in each season, the food items that require lower energy cost for their acquisition ought to be those most consumed.

2. STUDY AREA

The study was conducted over a 5,722 ha area at Os Montes do Invernadeiro Natural Park, NW Spain (UTM 29T 064633-643 and 467462-472). The topography of the area is mountainous with steep slopes. The altitude varies between 880 m and 1,707 m (Barja 2001). The climate is continental, with cold winters and hot summers. The annual average temperature and precipitation were 1,185 l m^{-2} and 10.4°C, respectively during the study period.

The study area occupies a transitional zone between the Mediterranean and Eurosiberian regions (Castroviejo 1977). This is manifested by the alternation between Mediterranean plant communities and relict Atlantic forests (Castroviejo 1977). Scrubland dominates the plant community, mainly heather (*Erica australis* L.), prickled broom (*Pterospartum tridentatum* L.) and sandling (*Halimiun lasianthum* Lam.). Original deciduous forest subsists in the valleys and along watercourses, and is formed mainly by associations of oak (*Quercus robur* L.), birch (*Betula celtiberica* Rothm. & Vasc.), holly (*Ilex aquifolium* L.) and rowanberry (*Sorbus aucuparia* L.) (Barja 2001). Large extensions are occupied by repopulated forests of Scot pine (*Pinus sylvestris* L.). The fauna is diverse, including carnivores such as the otter (*Lutra lutra* L., 1758), badger (*Meles meles* L., 1758), European polecat (*Mustela putorius* L., 1758), stoat (*Mustela erminea* Linnaeus, 1758), European common weasel (*Mustela nivalis* L., 1766), genet (*Genetta genetta* Linnaeus, 1758), wildcat (*Felis silvestris* Schreber, 1775), red fox (*Vulpes vulpes* L., 1758), and a large population of wolves (*Canis lupus* L., 1758) (Barja 2001).

3. MATERIAL AND METHODS

3.1. Collection and analysis of scats

Scats were collected monthly from July 2004 to June 2005 by establishing transects along roads. The use of roads by the European pine marten and other carnivores (wolf, red fox, wildcat) has been reported; these species frequently defecate on them, the scats acting as scent-marks (Pulliainen 1982, Robinson and Delibes 1988, Barja *et al.* 2005, Barja 2005a). Twenty-five transects 300 m in length were inspected every month. In order to include the territory of several individuals, and to ensure that the samples were representative, transects were established in four different zones of the study area set far apart from one another. In each

zone the transects were separated by at least one kilometer. Transects were established in optimal zones for the species, taking into account the results obtained in the study area on habitat selection and distribution in this species (Barja 2005b).

Pine marten scats were differentiated from those of other sympatric carnivores (wildcat, red fox and wolf) by their size and shape. To rule out the presence of the stone marten (*Martes foina* Erxleben, 1777) in the study area, 90 fresh scats were collected in the four zones where the transects were later set out. The DNA in the fecal residues was then identified using the PCR-RFLP technique (Ruiz-González *et al.* in press): all sampling were of European pine marten. Camera traps (155 camera-traps per night) were also set up, and visual surveys and searches for dead animals also made, resulting in the presence of the stone marten in the area being ruled out.

The scats were cleaned in the laboratory following conventional procedures (Reynolds and Aebischer 1991). After drying, the different macroscopic components were separated, weighed and identified. The hairs, teeth and bones found were used to identify the prey items. The cuticle patterns of the hairs were compared to those in reference manuals (Faliu *et al.* 1980, Teerink 1991) and with reference hairs collected in the study area. Their macroscopic characteristics were also compared with those in a museum collection (Museo Nacional de Ciencias Naturales of Madrid).

The date of collection, UTM grid position, and the age of all collected scats were noted. Scat age: fresh (deposition-4 days old), medium age (5 days-1 month old) and old (> 1 month old) provided an estimate of the defecation date and allowed the seasonal variation of the diet to be analyzed. Fresh feces were characterized by their strong smell, the presence of a layer of mucus, and the lack of signs of dehydration. Medium-age feces had lost their scent and the layer of mucus, but maintained their characteristic shape. Old feces had no smell and had lost their characteristic shape. To analyze seasonal variation, the months of the year were grouped into seasons: spring (April–June), summer (July–September), autumn (October–December) and winter (January–March). To avoid the replication of samples, scat positions were noted using a global positioning system (GPS) apparatus.

When the species diet is studied by analyzing the scats, it is necessary to consider that the relative importance of the food types changes during the digestion process of the predator (Lockie 1960). Therefore, the obtained proportion of each food type in the dry scats is not real. Thus, to correct this it is necessary to use some correction factors (fresh weight of a food multiplied by the dry weight of the remains of this food type found in the scats). This methodology was proposed by Lockie (1960) in a study conducted in European pine marten, where the weight variation of the different food types during the digestion process of this species were analyzed. In the present study, to estimate the ingested biomass of each food type, we multiplied the dry weight of each food type by its correction factor. The following Lockie's correction factors (1960) were used: small mammals – 22, rabbits and hares – 43, other large and medium-sized mammals – 61, birds – 37, beetles – 5, butterflies and moths – 12, berries – 14, and lacertids – 8.5.

3.2. Live trapping of small mammals

Live trapping is the method that reflects sufficiently the number of small mammals in an area (Gurnell and Flowerdew 1990, Luiselli and Capizzi 1996, Powell and Proulx 2003). To determine the abundance of small mammals in the study area, a live trapping campaign was undertaken each season (August 2004, November 2004, January 2005 and May 2005) in the most representative habitats of the study area (deciduous forest, mature pine forest and brushwood) (Barja 2005b). In each habitat, three trapping sites far from one another were selected. A grid containing 25 Sherman traps, separated by 10 m and covering an area of 0.25 ha, was set up at each sampling point. In each habitat the traps were left out for 3 consecutive nights and checked every 12 h. The total effort was 2,700 traps per night. Bread impregnated with oil was used as bait. All animals captured were identified and the date noted.

3.3. Data analysis

To determine the relationship between the consumption of small mammals by the pine martens and their seasonal abundance, the number of scats with the small mammal remains every season (*ObsF*) was quantified. Since the scat number collected in each season was different (Spring: 67, Summer: 56, Autumn: 54, winter: 32), the number of scats with the small mammal remains every season was corrected (*ObsF**: corrected number of scats with small mammal remains):

$$ObsF^* = ObsF \; x \; I_c \quad (1)$$

$$I_c = \bar{N} / N \quad (2)$$

where I_c is the applied correction index, N is the total number of scats analyzed per season, and $\bar{N}$ the mean number of scats analyzed per season.

The expected percentages of scats with small mammal remains (*ExpF%*) in each season were calculated according to the following formula:

$$ExpF\% = A_s \; x \; 100 / A_t \quad (3)$$

Where A_s is the seasonal number of captured small mammals and A_t the total number of captured small mammals in the study area.

The expected frequencies (*ExpF*) of scats with small mammal remains per season were calculated with the following formula:

$$ExpF = \sum ObsF^* \; x \; ExpF\%/100 \quad (4)$$

The Shannon-Weaver diversity index (Shannon and Weaver 1949) was used to calculate dietary diversity from the observed frequencies in each season.

Since the variables were not normally distributed, they were analyzed by non-parametric statistical tests. The χ^2 test was used to test the differences between the number of scats with small mammal remains per season (*ObsF**) and the expected number of scats with the small mammal remains per season (*ExpF*). In contingency tables where more than 20% of the expected frequencies were <5, the Monte Carlo's exact test was used. Yates' correction was used for 2 × 2 tables. Significance was set at $\alpha < 0.05$.

4. RESULTS

A total of 209 scats were analyzed. Mammals were found to be consumed over other prey types (50% of the ingested biomass), followed by fruits (28%), birds (21%), insects (0.8%) and reptiles (0.2%). Rodents were the most important mammal prey, accounting for 65% of the ingested biomass of mammals, followed by insectivores (22%) and carrion (roe deer, wild boar and badger) (13%). The differences in the predation frequencies with respect to the different mammal species were significant ($\chi^2 = 67.6$, d.f. = 2, 121, $P < 0.001$). Of the mammals taken, small mammals (rodents – except squirrels – and insectivores) made up 83% of the ingested biomass, mainly *Apodemus* sp. (46%), followed by *Glis glis* L. (11%), *Talpa occidentalis* Cabrera (7%) *Clethrionomys glareolus* (7%), *Crocidura* sp. (6%), *Neomys* sp. (6%), *Sorex* sp. (6%) *Arvicola* sp. (4%), *Eliomys quercinus* L. (3%), *Microtus* sp. (2%), *Mus* sp. (1%) and other not identified insectivores (1%) ($\chi^2 = 253.5$, d.f. = 11, 106, $P < 0.001$). The rowanberry (*S. aucuparia*) made up some 96% of the ingested fruit biomass.

During the trapping period, 201 small mammals were captured. The wood mouse (*A. sylvaticus*) was the most abundant species (95%), followed by the yellow-necked mouse (*A. flavicollis*) (3%), the pygmy shrew (*Sorex minutus* L., 1766) (1%), the greater white-toothed shrew (*Crocidura russula* Hermann) (0.5%), and the garden dormouse (*E. quercinus*) (0.5%). The differences between the different species captured were significant ($\chi^2 = 716.8$, d.f. = 4, 201, $P < 0.001$). The abundance of small mammals varied from one season to another. The greatest abundance of small mammals occurred in autumn (64.4 individuals ha^{-1}), followed by summer (22.2 individuals ha^{-1}), winter (3.6 individuals ha^{-1}) and spring (0 individuals ha^{-1}) (Fig. 1) ($\chi^2 = 16.3$, d.f. = 3, 201, $P < 0.001$).

The seasonal consumption of small mammals was not related to their abundance (Fig. 1). The differences between the expected (*ExpF*) and observed (*ObsF**) seasonal frequencies were significant ($\chi^2 = 16.3$, d.f. = 3, 105, $P < 0.001$). In spring and winter, when the abundance of small mammals was low, the pine marten showed a preference for this type

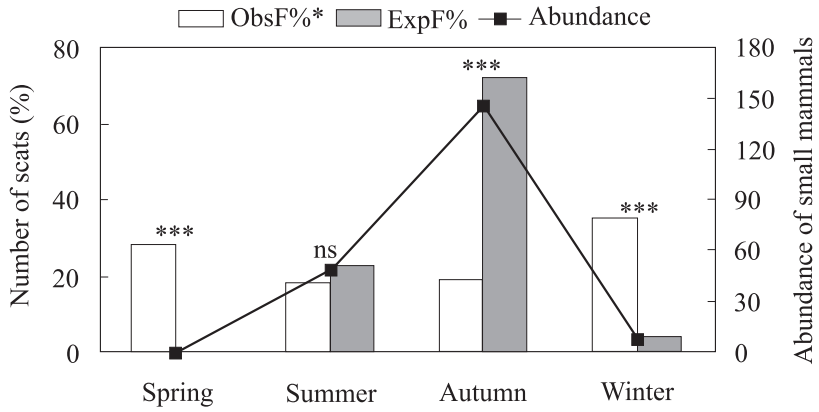

Fig. 1. Percentage of scats of European pine marten with small mammal remains (*ObsF%**, equation 1) per season, expected percentages (*ExpF%*, equation 3) and abundance of small mammals. The expected percentages of scats with small mammal remains (*ExpF%*) in each season were calculated multiplying the seasonal number of captured small mammals (A_s) × 100 and related to the total number of captured small mammals (A_t) (see method section). *** – P <0.001, ns – P> 0.05.

of prey (spring: 53% of the ingested biomass, winter: 60% of the ingested biomass) (Spring: χ^2 = 789.6, d.f. = 1, 30, P <0.001; winter: χ^2 = 243.0, d.f. = 1, 38, P <0.001). In summer, the consumption of small mammals was related to their abundance in the environment (24% of the ingested biomass), with no significant differences between observed and expected frequencies (χ^2 = 1.0, d.f. = 1, 20, P> 0.05). In autumn, although the abundance of small mammals was at its highest, the pine marten did not increase its predation of them (31% of the ingested biomass) (Fig. 1) – in fact it showed a preference for rowanberries (χ^2 = 41.0, d.f. = 1, 21, P <0.001).

The greatest dietary diversity and the lowest dominance values were obtained in spring (Shannon-Weaver index H = 1.37, Dominance D = 0.28) and winter (H = 1.33, D = 0.32); the lowest diet diversity and the highest dominance values corresponded to autumn (H = 1.01, D = 0.44). In summer, the food diversity diminished (H = 1.21, D = 0.34).

5. DISCUSSION AND CONCLUSIONS

In the study area, the diet of the European pine marten was varying, confirming the species to have an flexible feeding character and to be an efficient predator of small mammals. The species also appears to detect easily the birds' nests and young squirrels, and to take rowanberries before they fall. Mammals, however, were found to be the main prey, in agreement with what is indicated by other authors (Pulliainien 1980, Marchesi and Mermod 1989, Clevenger 1993, Jędrzejewski *et al.* 1993, Zalewski *et al.* 1995). The predator mainly focused on the genus *Apodemus*, the most abundant in the study area and a fundamental resource for this and other medium size carnivores such as the wildcat (I. Barja – unpublished). As this prey (mainly *A. sylvaticus*) is basically found in forests, it indicates a preferential use of this habitat type by the pine marten in the study area. This has been mentioned in previous studies conducted in the same area (Barja 2005) and in other parts of Europe (O'Sullivan 1983, Marchesi and Mermod 1989, Brainerd 1990).

A specialization towards taking small mammals was noticed; these preys were taken selectively although present in low densities. When a prey type is consumed with a higher frequency than that expected for its abundance, this reveals a behavior typical of a specialist species that does not change its food preferences even when that food is harder to find (Holling 1959, Begon *et al.* 1986, Jędrzejewski and Jędrzejewska 1993, O'Donoghue *et al.* 1998). In the present study, the pine marten showed no reduction in its preference for small mammals – mainly *Apodemus* sp. – in the sea-

sons when they were more scarce (winter and spring), even though squirrels, different forest birds, insects, reptiles and carrion (roe deer and wild boar, *Sus scrofa* L.) were all available. This absence of a preference change may in part be due to the weakness of small mammals during the colder months. The winter of 2004–2005 was especially cold and dry in the study area, and influenced the physical conditions of the small mammals caught during the trapping period. This reduced the effort required of the predator to pursue and capture them. In autumn, when the abundance of small mammals in the study area was at their highest, the pine marten did not increase its predation of them. In fact it showed a preference for rowanberries, probably because this food provides an advantage in terms of energy optimization. When the pine marten consumes the berries the manipulation time diminishes (there is no pursuit phase as there is with animal prey), meaning a better energy balance for the predator (Kacelnik and Bernstein 1994).

The results of this study show that the feeding strategy adopted by the European pine marten is intermediate between that of an opportunist and specialist predator. Previous studies have classified the species as a strict opportunist. Small mammals constitute its main prey and they are consumed although present in low densities. The pine marten probably has a crucial influence on small mammal populations in forest areas, especially on the genus *Apodemus*.

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